CODLING MOTH* SEX PHEROMONE COMPONENTS AND RELATED COMPOUNDS. AN EVALUATION OF THEIR POTENTIAL AS MATING DISRUPTANTS

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* Lepidoptera: Tortricidae: Olethreutinae.

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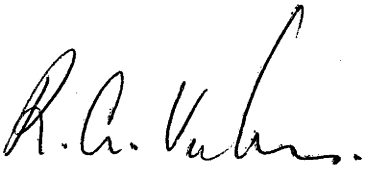
CONTENTS

6. APPENDICES (cont).

DECLARATION

The work presented in this thesis is entirely my own unless otherwise indicated.



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ABSTRACT

Multi-component blends of (*E,E*)-8,10-dodecadien-1-ol (EEOH), dodecanol (12OH), tetradecanol (14OH) and in some trials (*E*)-9-dodecadiene-1-ol (E9-12OH), were evaluated in terms of their ability to attract males to traps, disrupt mating and influence male behaviour in the field and in laboratory bioassays. In no respects were the blends superior to EEOH alone.

Calling virgin females released pheromone at a mean rate of 0.13 ng/h. Analyses of the effluvia revealed, *inter alia*, an homologous series of saturated alcohols present in quantities that were mainly independent of EEOH quantity. The same alcohols were found in collections from non-calling females, males, the body scales of virgin females and hexane used as a solvent in the collection procedure.

On the basis of the trapping and mating disruption trials, observations of male behaviour in the field and laboratory and results from effluvia analyses, it was concluded that the saturated alcohols 12OH and 14OH had no behavioural activity, were probably not present in the effluvia and their addition to mating disruption blends was not justified.

The Shin-Etsu dispenser used for most of the mating

disruption trials is able to maintain the integrity of its contents and of its effluvia despite prolonged exposure to the degradative effects of sunlight (ultra-violet light) and oxygen. Pheromone release rates were considerably slower from dispensers exposed to sunlight than they were from those that had been shaded or not exposed to any light. However the reason for these differences was not established.

Male activity within cages in air permeated with EEOH was significantly lower than that in pheromone-free air, suggesting that one of the mechanisms by which mating disruption might be achieved is through suppression of male activity.

The ability of (*Z*)-8-dodecenyl acetate (Z8-12Ac) to suppress trap catch diminished as the distance between its source and that of the attractant increased. Neither this compound nor (*E,E*)-8,10-dodecadienyl acetate (EEAc) were effective in suppressing trap catch when released as a background treatment.

By delaying the mating of females, a possible consequence of imposing mating disruption treatments in addition to that of preventing mating, the net reproductive rate was reduced by 45% in females mated 4 days after emergence compared with those mated on the day of emergence. Delaying the mating of males had no significant effect on

the reproductive rate of the females they had inseminated.

1. INTRODUCTION

Codling moth is an important and sometimes the key pest in many pome-fruit growing regions of the world. Its control is conventionally achieved with insecticides and it is frequently required that populations be maintained at or below a level commensurate with 1-2% fruit damage. However increasing public concern about the harmful effects of insecticide residues on human health and the environment, together with the recently-realised potential of codling moth to develop resistance to the most commonly used insecticide for its control, azinphos methyl, has introduced some urgency into the need to find alternative methods of controlling the species.

Synthetic sex pheromones are considered to have some promise as an alternative to insecticides, either indirectly as monitors of populations, so enabling other control measures to be more precisely timed, or directly as mating disruptants. It is with the latter application that the research in this thesis is concerned.

Roelofs (1978) postulated that the quantity of synthetic pheromone required to elicit the sequence of male behaviours that culminates in location of the pheromone source increases the more the pheromone blend departs from the natural composition. An implication of this 'Threshold Hypothesis' is that the closer a mating

disruption blend resembles the natural blend the more efficacious it will be.

In a review of attempts at mating disruption with complete and incomplete pheromone blends for several Lepidopterous species, Minks and Carde (1988) found that evidence to support the hypothesis was equivocal, but nevertheless concluded that "... it would appear unwise to pursue the costly development of formulations that rely on less than the natural blend, unless field trials have verified disruptive efficacy comparable to (or magnified over) the natural blend and the quality of disruptant needed is cost effective."

Field trials in Australia with the major codling moth pheromone component, (*E,E*)-8,10-dodecadiene-1-ol (EEOH) (Rothschild and Vickers 1982) indicated that although area treatments with the compound were often able to significantly reduce catches at traps baited with the same material, on no occasion was there any evidence that the incidence of mating in the wild female population had been affected by the treatments. Results elsewhere had been variable (Rothschild 1982) and it was postulated (Bartell *et al* 1988) that on the basis of the demonstrated biological activity of dodecenol (12OH) and tetradecenol (14OH) (Bartell *et al* 1988), addition of these components to EEOH might make a more effective mating disruptant.

The failure of EEOH alone to disrupt mating under Australian conditions and the findings of Bartell *et al* (1988) provided the rationale for the research described in this thesis. It is primarily an account of studies to evaluate the ability of multi-component pheromone blends to influence male behaviour and disrupt mating.

Several compounds, in some instances bearing a structural similarity to EEOH, have been shown to have an inhibitory effect on catch at pheromone traps baited with EEOH when released from within the trap. Two of the most promising of these compounds were evaluated as mating disruptants.

Trials were also undertaken to determine:

1. the composition of the effluvia from female codling moth;

2. the ability of the dispensing system used for most of the disruption studies to maintain the quality of pheromone delivered into the atmosphere;

3. the effect of delayed mating, a possible consequence of mating disruption treatments, on net reproductive rate.

In summary, the aims of the research described in this thesis were to:

1. assess the potential of multi-component pheromone blends and two related compounds known to inhibit trap catch to disrupt mating;

2. identify the components in female effluvia;

3. investigate the stability of pheromone released from the dispensing system used in most of the mating disruption trials.

4. determine the effects of delayed mating on net reproduction rate of codling moth.

2. LITERATURE REVIEW

2.1 BIOLOGY AND ECOLOGY

Codling moth (*Cydia pomonella* (L) (Tortricidae) is thought to have originated in the Palearctic region, where it probably co-evolved with the deciduous fruits on which it is wholly dependent (Geier 1981). The primary hosts of codling moth (apples, pears and quinces), all belong to the Rosaceae family. Other less frequently attacked fruits such as peaches and apricots are considered secondary hosts, whilst infestations of persimmons, pomegranates and oranges are probably accidental (Bovey 1966). Infestations in walnuts (Juglandaceae) may be caused by a distinct race of codling moth (Barnes *et al*, 1966, Barnes 1991).

The species has one to three generations a year, depending upon locality. In response to shorter day length (Riedl & Croft 1978), final instar larvae from the last generation in a season and a small proportion from preceding generations overwinter as mature larvae, pupate in late winter/early spring and emerge as adults from spring to early summer. A very small proportion of overwintering larvae may not pupate for another 12 months (Geier *et al* 1983). In areas where the species is multivoltine, the generation cycles begin to overlap before mid-summer (Geier *et al* 1983).

Mating is mediated by a female-produced sex pheromone, the existence of which was reported by Barnes *et al* (1966). Visual and tactile cues may also be involved in mate-seeking behaviour (Castrovillo & Carde 1980). Pheromone production reaches its maximum in three-day old females (McDonough *et al* 1969, Benz 1970), corresponding with the pre-oviposition period (Geier 1981). Nevertheless mating can take place on the first day after emergence. In a laboratory study of codling moth mating behaviour Howell *et al* (1978) found that 24 h old virgin females mated as readily as those that were 72-96 h old. The proportion of males mating for the first time was highest amongst those that were one day old than at any other age tested (2-7 d).

Eggs are deposited singly on or near the fruit (Wearing *et al* 1973) and their scattered distribution probably reduces predator pressure and limits the incidence of multiple attack on individual fruit (Jackson 1979). Generally only one larva survives to maturity in instances of multiple attack. The others succumb to cannibalism, premature shedding and/or decay of the fruit (Geier *et al* 1983). First instar larvae superficially mine the fruit, leaving 'stings' if the attack progresses no further, but otherwise creating 'deep entries' as they tunnel towards the centre of the fruit. After passing through four further instars the mature larva leaves the fruit to pupate in sheltered

sites on the tree. A small proportion may pupate in the ground beneath the tree.

2.2. PEST STATUS AND CONTROL

Whilst codling moth may cause significant damage in areas where it is univoltine, it is of greatest significance as a multivoltine pest of late maturing apples (Geier 1981, Riedl & Croft 1978). In western USA, South Africa and Australia codling moth has assumed key pest status, whilst in eastern USA, Europe and New Zealand it is part of a broader complex of lepidopterous pests that includes leaf-rolling tortricids. In all areas codling moth is conventionally controlled with broad spectrum insecticides, which have led to a reduction in the predator complex and the creation of secondary pest problems such as phytophagous mites. Azinphos methyl, an organophosphate, is currently the most commonly used insecticide for controlling codling moth. When used correctly damage can normally be maintained within the 1-2% level demanded, for example, by many commercial orchardists in Australia. However there is now evidence of codling moth having developed resistance to azinphos methyl in America (Welter *et al* 1991) and Australia (G Thwaite, NSW Dept. Agriculture, personal communication 1991), which may limit its use in the future.

Attempts at biological control of codling moth have

generally failed (Clausen 1978, Geier 1981), in large part because during most of their developmental period the larvae are virtually inaccessible to attack by parasites. So too are the overwintering larvae in their hibernaculae that are often spun up within deep fissures on the tree trunk and limbs. Furthermore, secondary parasites may cause heavy mortality amongst the over-wintering primary parasite population (Clausen 1978). The release of sterile males as a control measure has been exhaustively tested, particularly in the USA, but has had very limited success (Proverbs *et al* 1977).

The granulosis virus CpGV, first discovered in 1963 (Tanada 1964), has shown considerable promise as a control agent for codling moth and is likely to be commercialized in the near future (Falcon and Huber 1991). The prospects for codling moth control with the bacterium *Bacillus thuringiensis* do not look so promising (Falcon and Huber 1991), in part because the feeding behaviour of first instar larvae results in them having very little contact with deposits of the bacterium on fruit or foliage. (Undorf and Huber 1986, cited in Falcon and Huber 1991).

2.3 MATING BEHAVIOUR

2.3.1 Calling and mating.

The onset and duration of female calling and male response are regulated by environmental cues, particularly temperature and light. Whilst these behaviours generally take place during the first hour after sunset (Wong *et al* 1971, Mani *et al* 1974), Castrovillo & Carde (1979) found that under controlled laboratory conditions a drop in temperature from 23°C to 16°C three hours before the onset of scotophase resulted in the calling peak shifting to the photophase.

There was no corresponding shift in male response to pheromone, for which sex the relationship with temperature may be more complex. However there would be no advantage in females shifting their calling period in response to environmental cues if males were unable to respond and it seems likely that males have a 'broad-band' response.

Calling and mating may begin on the first day after emergence, although pheromone production probably reaches its maximum on the third day, corresponding with the pre-oviposition period (Geier 1981). Once coupled, females are no longer attractive to males (Howell *et al* 1978) and they may possibly produce compounds that deter sexually-responsive males (Rothschild 1982).

Under field conditions the majority of females mate only once. Of those collected over an 8-year period from terpinyl acetate feeding lures (Rothschild & Vickers, unpublished data), 21% (458) contained two or more spermatophores, 68% (1483) contained one and 11% (240) were unmated. Of females collected in a light trap 13% (178) contained two or more spermatophores, 76% (1041) contained one and 11% (148) no spermatophores. Gehring & Madsen (1963) found that the incidence of multiple mating was higher towards the end of the season, and in a laboratory study Howell *et al* (1978) reported a significant increase in the incidence of second matings on the 6-7th days after emergence. As oviposition had almost been completed by this stage, the implications of this finding were not clear.

Males too are capable of mating several times. Howell *et al* (1978) showed that one spermatophore a day could be passed for 3-5 consecutive days. However spermatophore size decreased over this period and mating took longer to accomplish, leading the authors to comment that 'codling moth is not well adapted to polygyny'.

2.3.2. Visual cues and mating

Close-range cues. On the basis of laboratory studies in which the mating success of males with none, one or both antennae removed was compared, Fluri *et al* (1974)

concluded that even at close range (two male and two females were paired in 2.5 l containers) olfactory cues were required for successful mating. Whilst there was no significant difference between the levels of mating recorded in entire males and those with one antenna, removal of both antennae reduced mating to almost zero. From this result it can be inferred that in the absence of any olfactory input visual cues were not sufficient to bring about successful mating.

A somewhat contrary result was obtained by Hutt & White (1977). They determined that visual stimuli contributed to male copulatory response in a laboratory trial where levels of mating in clear plastic bags screened on one end (White and Hutt 1971) were compared in males that were either antennectomized, blinded or antennectomized and blinded. A control group was left untreated. There was little difference between the first and second treatments (46% and 59% respectively), but only 19% of the males that were both antennectomoized and blinded had mated. In the control group 87% had mated.

Although the results indicated that vision played a significant role in male copulatory behaviour, the level of mating achieved in the absence of both visual and olfactory cues suggested to the authors that under these conditions 'a state of physiological receptiveness on the part of the male can occur and a successful mating be accomplished'.

Gehring & Madsen (1963) found that in close proximity males and females mated as readily in constant darkness as they did under natural light and dark conditions and concluded that visual stimuli were not required for successful courtship.

Measurements of mating frequency in moths confined in such close proximity are somewhat artificial and bear little resemblance to events in nature. A more realistic approach to the role of visual cues was adopted by Castrovillo & Carde (1980), who examined the effect of a visual cue (a dead female) on males flying towards a pheromone source in a wind tunnel. Whilst the time spent orienting towards the source or in contact with the platform (from which the pheromone was released and on which the cue rested) was not influenced by the presence or absence of the visual cue, significantly more time was spent walking, wing fanning and attempts at copulation in the quadrant containing the cue than in the quadrant containing the pheromone source. As this was true even when the female was placed upwind of the pheromone source, it would appear that visual cues take precedence over olfactory ones once visual contact has been made. However the results are contradicted by statements made earlier in the account of the trials, where it was said that 'females without synthetic pheromone were unattractive to males' and 'when males had reached females in the presence of pheromone and

flask air (ie pheromone) was stopped, the males soon left the platform with no reorientating'.

Long-range cues. Although codling moth is considered a sedentary species, mark-recapture studies indicate that a small proportion of males disperse beyond orchard boundaries. Visual cues appear to influence the direction and distance travelled. Howell & Clift (1974) recovered males up to 8 km from their release point, but only in traps located in isolated host plants and not in those placed on poles or non-host plants. Mani *et al* (1974) and Mani & Wildbolz (1977) however, recaptured males at traps in both host and non-host vegetation and concluded that the prominence of the plant in relation to its surrounding vegetation and topography was important.

The distance over which pheromone-releasing females are able to attract males is not known, but in any case will probably vary with meteorological conditions, particularly wind speed. In their native habitat, where host trees occur in isolation rather than as a man-made monoculture, it is conceivable that males respond initially to visual and/or olfactory cues presented by potential host plants, in which females are more likely to be found. Under orchard conditions inter-tree distances are probably less than the 'active space' of the pheromone plume and the use of visual cues to locate females may not be necessary. Under these circumstances

olfactory cues alone should suffice to allow males to locate calling females.

2.4. PHEROMONE IDENTIFICATION

The existence of a female-produced odour that was attractive to male codling moth was suspected as early as 1837 (Jacobson 1965), but more than a century passed before the first attempts were made to determine the chemical nature of the attractant. Butt & Hathoway (1966) demonstrated that males were attracted to extracts of female codling moth abdominal tips. In a survey of aldehyde and nitrile compounds Butt *et al* (1968) identified several nitrile attractants, while McDonough *et al* (1969) found evidence of an epoxide group, which was subsequently identified as (2*Z*, 6*E*)-7-methyl-3-propyl-2,6 decadienol (McDonough *et al*, 1972a). Field tests of these compounds indicated that while the nitriles were mildly attractive, the epoxide failed to catch (McDonough *et al*, 1972b).

Roelofs *et al* (1971) used antennal responses to gas chromatograph fractions of gland extracts and subsequently to a series of synthetic compounds to determine that EEOH was the pheromone. The sexual stimulation elicited in laboratory bioassays by the compound was also evident in the field, where it proved very attractive to males.

George & McDonough (1972) claimed that there were several components in the pheromone. However on the evidence of trap captures there was nothing to suggest that EEOH alone was inferior to the pheromone released by virgin females. Batiste *et al* (1973) found that catches at traps baited with either 5 virgin females or EEOH were similar, whilst Madsen & Vakenti (1972) and Culver & Barnes (1973) reported significantly higher catches at EEOH-baited traps than at virgin females.

Evidence of other behaviourally-active components in the female sex pheromone was first provided by Bartell & Bellas (1981). They compared male response to female extract and to synthetic EEOH and found that the median response dose for the extract was significantly lower than it was to EEOH. In terms of probit regressions of sexual response on EEOH concentration, the extract was 1200-fold more potent than synthetic EEOH, suggesting that there were components in addition to EEOH that were eliciting a behavioural response.

In the same report further evidence for the existence of one or more additional components was provided in a comparison of male responses to synthetic and natural EEOH, female extract with and without natural EEOH and female extract in which the natural EEOH had been replaced with synthetic EEOH. The percentage response to whole extract was three times greater than it was to both natural and synthetic EEOH presented in

concentrations similar to that contained in the whole extract. This result was attributed to the presence of one or more additional behaviourally-active components in the whole extract.

Einhorn *et al* (1984) provided evidence supporting Bartell & Bellas, with the finding that gland extracts containing 0.1-100 ng EEOH were 10 times more active in terms of the proportion of males landing than EEOH alone in wind-tunnel bio-assays.

In the same study an analysis of gland contents using a combination of gas chromatography (GC), gas chromatography/mass spectrometry (GC/MS) and microchemistry revealed 13 components (Table 1). Arn *et al* (1985) analysed both the gland contents and effluvia. Whilst the results of the gland analysis (Table 1) were in broad agreement with those of Einhorn *et al*, a surprising (but unremarked) feature of their effluvia analysis was that the proportions of hexadecenol (16OH) and octadecenol (18OH) were at least four times higher than had been found in the gland. It is unlikely that these components are synthesized by the female during the calling period and as volatility decreases with increasing carbon-chain length, one might expect relatively more of these saturated alcohols to be found in the gland rather than in the effluvia.

In addition to analysing the effluvia, Arn *et al* also

found evidence of dodecan-1-ol (12OH) having biological activity. In wind-tunnel bio-assays EEOH used at optimum concentrations elicited landing of males at a rate equal to or better than that achieved with calling females. At this concentration the addition of 12OH did not influence the performance of EEOH. However landing rates adversely affected by the use of sub-optimal dosages of EEOH were restored to optimal levels by the addition of 12OH. Under these conditions 12OH increased the percentage of males undertaking upwind flight but had no influence on the preceding behaviours, excitation and take-off.

Bartell *et al* (1988) also identified 12OH as having behavioural activity, but only when presented in conjunction with both EEOH and tetradecanol (14OH). They were able to elicit in males levels of sexual activity similar to those achieved with female extract in their earlier bioassays (Bartell & Bellas 1981) by adding 12OH and 14OH to synthetic EEOH. The addition of one or the other saturated alcohol did not significantly increase activity levels over those achieved with EEOH alone.

Einhorn *et al* (1986) confirmed the findings of Arn *et al* (1985) that 12OH synergised EEOH in terms of the percentage of males that flew upwind when EEOH was at sub-optimal levels. In the same study the influence of 12OH and other secondary components on male trap catch in the field was tested. None were found to increase

trap catch over that achieved with EEOH alone. Causse *et al* (1988) subsequently found that while a synergistic effect could not be demonstrated for 12OH when the traps were placed three metres apart, at 20 metre spacing there was a significant increase in catch.

Component	% found in		
	Gland[1]	Gland[2]	Effluvia[2]
(E,E)-8,10-12:OH	60.5	56.1	42.2
(E,Z)-8,10-12:OH	3.0	2.1	2.3
(Z,E)-8,10-12:OH	-	0.3	<0.005
(E,E)-8,10-12:ALD	1.0	0.5	-
(E,E)-8,10-12:Ac	-	0.1	-
(E)-8-12:OH	0.5	-	-
(E)-9-12:OH	2.5	5.3	6.2
10:OH	-	0.1	1.2
12:OH	18.5	26.7	27.2
14:OH	8.0	5.3	3.9
16:OH	6.0	1.1	7.8
18:OH	-	2.1	9.3
18:Ac	-	<0.005	-
20:Ac	-	<0.005	-

Table 1. Components and their proportions found in the pheromone gland and effluvia of codling moth. (1 = Einhorn *et al* 1984, 2 = Arn *et al* 1985).

The findings of Arn *et al* (1985) and Einhorn *et al*

(1986) concerning the role of 12OH as a synergist are remarkably similar to those of Linn *et al* (1986), who in their assessment of the functional role of pheromone components found that 12OH, a minor component of the pheromone of the oriental fruit moth (*Cydia molesta*), was synergistic only when another component, Z8-12OH, was present in less than optimum concentrations.

Thus claims of a behavioural role for 12OH are somewhat ambiguous. Rumbo (personal communication, 1989) found evidence for the inhibition of some antennal cells sensitive to EEOH by 12OH and suggested that the synergistic effect of 12OH when EEOH concentrations were excessive might be a consequence of inhibition, although this would not explain similar effects when EEOH concentrations were too low.

2.5. MATING DISRUPTION WITH EEOH

Codling moth has been the subject of many mating disruption trials, comprehensive reviews of which have been provided by Rothschild (1982) and Vickers and Rothschild (1991). Most field trials have used either broadcast formulations or hand-placed discrete dispensing stations to release the major pheromone component, EEOH. There have also been a small number of laboratory trials with EEOH. Several non-pheromone components have been evaluated as disruptants in the field.

2.5.1. Field studies

2.5.1.1 Broadcast formulations

Broadcast formulations in the form of microcapsules or hollow fibres release pheromone from a large number of point sources throughout the orchard and are assumed to provide a more uniform distribution of pheromone than can be achieved with discrete dispensing stations. Moffit (1974) released EEOH from National Cash Register (NCR) microcapsules applied to 0.3-0.4 ha plots at rates equivalent to 7.4 g/ha. Captures of males at pheromone and virgin female baited traps were suppressed to a level 93-95% below those of the untreated check plots for 7 days, but by the eighth day suppression had fallen to 65%. A second application of EEOH at the same rate was effective in suppressing catches for only 14 days during the season and claims that the applications were responsible for a 90% and 60% reduction in larval and fruit damage respectively must thus be treated with some caution.

Mani *et al* (1978) used a knapsack sprayer to distribute a slurry of NCR microcapsules and water containing *ca* 1 mg EEOH/ml in 9 and 49-tree plots, the areas of which were not stated. The microcapsules contained 2% EEOH and 5% of the anti-oxidant di-tert pentyl hydroquinone in xylene and had a diameter of 50-200 µm. Catches at virgin

female and EEOH-baited traps were suppressed in the 9-tree plots by 75% in the first week, after which there was no effect. In the 49-tree plots catches were reduced by 93% and 43% respectively. No information about the release rate characteristics for microcapsules used in codling moth disruption trials is available. However similar NCR capsules containing gypsy moth pheromone (disparlure) tested over a 66 day period lost only a small proportion of the pheromone loading, most of it within the first 2 days (Caro *et al* 1977).

Chopped hollow polymer fibres release pheromone from their open ends at a rate determined by lumen diameter, whilst fibre length governs longevity. Moffitt (1978) reduced catches at virgin female baited traps by 82% over a 16 day period in 0.3-0.4 ha apple and pear blocks treated with aerially applied fibres releasing at a mean rate of 3 mg/ha/h. In a concurrent trial pheromone was released at 13 mg/ha/h and trap catch reduced by 87% over 16 days. Over two months the reduction exceeded 80%. With a similar release rate Moffitt reduced catches by 98% over one month later in the season in a 0.4 ha plot of apples. The same paper describes an attempt to provide seasonal protection to 0.4 ha pear blocks with three applications of chopped fibres in late April, May and June. Fibres were applied at a rate equal to *ca* 11 g/ha/treatment (1500 fibres/ha) and released pheromone at 7-10 mg/ha/h. At the end of the season-long trial fruit damage was estimated to be 0.5% in the pheromone

and insecticide-treated plots compared with 4-9% in the control plots.

Further trials by Moffitt & Westigard (1984), in which three pear orchards were treated over a two year period with three to five applications of fibres/season (10.6 g EEOH/ha/treatment) reduced male response to EEOH-baited traps in orchards where initial population densities were low. Where they were high, catches were not significantly different from those of the control. Fruit damage at harvest was within commercially acceptable limits and in both years was significantly lower than that sustained in the controls, but again only where initial population densities were low. Where they were high, damage in both years approximated 58%. In all orchards damage exceeded that of the standard insecticide-treated plots despite generally lower trap catches where pheromone treatments were used. Subsequent trials in New Zealand (Moffitt *et al* 1982) confirmed that where initial population densities were high, chopped hollow fibres were unlikely to keep codling moth in check.

2.5.1.2. Dispensing stations

In contrast to broadcast formulations, dispensing stations produce locally high levels of pheromone from discrete release points. Several types have been evaluated:

(1) *Hollow fibre tapes*: Pheromone is released from the open ends of polyester terephthalate fibres which are attached in parallel arrays to strips of adhesive tape. Release rate is determined by internal fibre diameter and the number of fibres. Carde *et al* (1977) suppressed trap catch by 100% and 96% at virgin female and EEOH baited traps respectively in a 1 ha plot treated with hollow fibre tapes placed at a rate of 25/tree. Each tape contained 10 fibres (length 1 cm, internal diameter 200 µm) loaded with 98% EEOH (97% pure) and 2% antioxidant. A slight increase in trap catch within the treated plot during the trial was thought by the authors to have been caused by a reduction in the rate of EEOH release, although Rothschild (1982) pointed out that as there was a coincidental rise in moths taken at control plot traps, a general increase in adult numbers may have contributed to the minor breakdown.

In Switzerland Mani *et al* (1978) placed 25 tapes, each with 25 fibres (300 µm diameter - internal or external not specified) throughout plots of 9 and 49 apple trees. Reductions in catches at virgin female and EEOH-baited traps varied between 85-88% in the smaller plots and 90-98% over 6 weeks in the larger ones. The area of the plots and the release rates were not specified for these trials, but on the basis of other data given in the paper release rates were estimated to be 4 mg/ha/h (Rothschild 1982).

Stenmark (1978) reduced catches by 98% at pheromone traps in 0.9 ha apple plots treated with 25 tapes/tree (18 fibres/tape), where the mean release rate over the three month trial period was *ca* 3 mg/ha/h. Fruit damage in this trial was unacceptably high and although release rates were considered by the author to have been inadequate, Rothschild (1982) suggested that as the treated area was so small, immigration of mated females may also have been a contributing factor.

(ii) *Rubber tubing*: Rubber tubing, commonly used as a substrate for trap baits, has also proved suitable as an evaporator for the much larger quantities of pheromone used in disruption trials. Loadings are easily varied depending upon the requirements of the researcher, but typically range between 20-100 mg/dispenser. The ends of the tube are sealed with metal clips or rings. Pheromone passes through the walls of the dispenser and thus release rates are influenced by the amount of pheromone within the dispenser and by temperature. Audemard *et al* (1977) attempted seasonal control of codling moth in a 0.7 ha orchard with EEOH dispensed from rubber tubing at rate of 13-30 mg/ha/h during the first generation. Total suppression of trap catch was achieved throughout the season and apparently fruit damage was significantly reduced. Treatments confined to the second generation failed to protect the fruit despite release rates equivalent to 100 mg/ha/h.

Rubber tubing has been tested extensively in Switzerland. Mani (personal communication, 1986) conducted disruption trials in 22 commercial apple orchards in eastern Switzerland between 1979-85. In trials over three years in a 0.9 ha isolated orchard in the upper Rhine Valley (Mani *et al* 1984), trap catch was totally suppressed, as was mating of tethered females. Larval populations were reduced and fruit damage was kept below the economic threshold of 2%. A single placement of 270 rubber tubing dispensers, each 7-8 cm long and loaded with *ca* 80 mg EEOH was made at the beginning of each season (mid May), giving an application rate equal to 24 g/ha/season. Loss rates, determined gravimetrically at weekly intervals, varied between 2.5 to 17.5 mg/ha/h. Mani (personal communication, 1986) also treated a 3.5 ha mature (12-15 years old) commercial apple orchard with one application of rubber tubing dispensers in May 1982 and 1983 and two in May and July of 1984. Dispensers were placed at 1/100 to 150 m^2 applications (with additional ones at the borders), equivalent to 25 g EEOH/ha in 1982 and 1983 and 40 g in 1984. Assessments were made of male catch at pheromone traps, mating of tethered females and the size of larval populations.

Results were compared with those of a nearby check orchard used for cider production which was not treated for control of codling moth during the trial. Fruit

damage in the treated orchard was kept below 2% in each year. Larval numbers at the end of the season preceding the first placement of pheromone were *ca* 600/ha in the treated orchard and *ca* 900/ha in the control. When the trial concluded (1984) the larval population was *ca* 1500/ha in the treated orchard but *ca* 22,000/ha in the control.

Catch was totally suppressed during 1982 in traps placed at a height of 1.7 m. In 1983 and 1984 additional traps were placed in the tops of the canopy and in border trees. Suppression of catch was again total in the lower internal traps, but numbers captured in traps along the border and in the canopy were said to be 'few' and 'many' respectively. A similar trend was evident with tethered females, where there was no mating in the lower placements but some in the tree tops, suggesting that pheromone levels in the upper canopy were too low to prevent mating.

Charmillot (1986) treated four Swiss orchards (total area *ca* 11 ha) with rubber tubing impregnated with 99% EEOH (99% pure) and 1% of the anti-oxidant BHT at a rate of 1.5 mg/mm of tubing. Two approximately equal placements were made, one at the beginning of the season (mid May) and another early in July. In three of the four orchards 40-44 g EEOH/ha/season was applied. In the fourth orchard the seasonal total was 84g/ha, made up of an initial 58 g and subsequent 26 g placement. Pheromone

sources were distributed so that 20-25% of the total quantity was placed around the borders and the remainder on internal trees. The surface area serviced by each dispenser ranged from 144-225 m^2 for the three orchards with the lower application rates to 38.5 m^2 for that with the highest rate. In all orchards release rates rarely dropped below 10 mg/ha/h, and then only at the very beginning and end of the season. Trap catches were suppressed by 92-99% in the treated orchards and fruit damage varied between 0-0.05%. No damage levels were given for the control orchards.

(iii) *Laminates* These dispensers were developed by Hercon, a subsidiary of the Health-Chem Corporation of America. They consist of several layers of laminated polymeric material with the active ingredient implanted and protectively sealed between outer plastic layers (Quisumbing & Kydonieus 1982). Pheromone migrates from the reservoir to the outermost layer at a rate determined by reservoir concentration, membrane thickness, polymer stiffness, codiffusants, molecular weight of diffusant and chemical functionality (Quisumbing & Kydonieus 1982). Laminates have been manufactured in forms suitable for hand application (in strips or squares) as well as for broadcasting ('flakes' or 'confetti').

The strip form was used by Charmillot (1986), who achieved a 99% reduction in trap catch and no measurable

fruit damage in a 9 ha apple orchard treated twice during the season (47.4 g and 23.8 g EEOH in May and July respectively). Larval populations, determined by counting larvae recovered from trap bands at the end of the season, averaged 0.03/band. No information on fruit damage or larval populations was provided for the control orchard. Similar results were achieved in earlier trials (Charmillot, personal communication 1986), although in 1985 a 0.1 ha orchard treated three times during the season with laminates containing 33.6 g EEOH/ha/application suffered 6.3% damage at harvest compared with 0.3% in the chemically-treated control plot. This occurred despite a trap catch suppression of almost 94%. An average of 43 larvae was recovered from each trap band in the pheromone-treated orchard, compared with 0.2/band in the control. Charmillot (personal communication, 1986) considered that the small size of the orchard and the late initial application of pheromone (ie after the first flight had begun) contributed to the failure.

2.5.2. Laboratory studies

The only mating disruption trials conducted in the laboratory have involved an assessment of mating levels of closely confined adults exposed to high concentrations of EEOH. Fluri *et al* (1974) exposed single pairs of moths in 1.2 l containers to rubber septa loaded with one mg of EEOH for up to 16 days. A

65% reduction in the level of mating was noted over that obtained in the controls. However it is possible that a greater reduction might have been obtained over a shorter period. It is likely that pheromone was lost by degradation and/or absorption onto the glass walls of the container, possibly causing pheromone concentrations to drop below the minimum effective level towards the end of the experiment.

In a similar trial Charmillot *et al* (1976) placed 5 pairs of adults in 1.2 l containers, through which air was passed but having first been directed over either one or three rubber septa, each of which was loaded with 1 mg of EEOH. Mating at the high and low loadings was reduced by only 38% and 28% respectively, despite loss rates which on the basis of work done elsewhere with similar dispensers and EEOH loadings (Maitlen *et al* 1976) was probably about 1.5 ug/septa/h.

It is clear that the sexual responsiveness of only a limited number of males is affected by their continuous exposure to relatively high levels of EEOH. If, as suggested by the evidence of Hutt and White (1977), visual cues can operate at close range regardless of the presence or absence of pheromone, it is hardly surprising that mating could not be entirely suppressed in these trials. As it is most unlikely such conditions could be achieved in the field, experiments of this nature provide little information about likely

disruption mechanisms. Furthermore, confining adults in such close proximity does not allow for the possibility of achieving mating disruption by influencing male orientation behaviour.

2.6 MATING DISRUPTION WITH OTHER COMPOUNDS

A number of compounds known to influence the ability of males to locate traps baited with virgin females or synthetic baits have been investigated as mating disruptants. Arn *et al* (1974) placed various compounds in traps together with EEOH baits and found that most of those that inhibited trap catch were straight-chain 12 or 13 carbon acetate compounds with a double bond at the 8 or 10 position. George *et al* (1975) undertook similar tests with ester and ether derivatives of EEOH and found that the most potent inhibitors were acetate, propionate, methyl and propyl compounds. However as Rothschild (1974) indicated, compounds that inhibit trap catch when placed within the same trap as the bait do not necessarily have the same effect when used as background treatments. This was confirmed by Arn *et al* (1974), who found that although (*Z*)-8 dodecenyl acetate (Z8-12Ac) totally suppressed catches at virgin female traps when dispensed from rubber septa in the same trap, it failed to do so when released as a background treatment at 6-25 mg/ha/h.

An acetate derivative of EEOH, (*E,E*)-8,10-dodecadienyl

acetate (EEAc) in quantities as low as 50 mg was shown by Hathaway *et al* (1974) to inhibit catches when placed in traps with either virgin females or synthetic baits. In wind tunnel tests, Preis *et al* (1977) found that males would not land at EEOH sources containing 10% EEAc. The compound was also shown to influence catch when dispensed from rubber septa as a background treatment at rates equivalent to 60-130 mg/ha/h in pear tree plots (Hathaway *et al* 1979). Over a period of 7 weeks, male captures at virgin female baited traps were reduced by about 98%. In another trial a 90% reduction over 7 weeks was achieved with EEAc released at the equivalent of 25 mg/ha/h.

Undecanol was shown by Charmillot (1980a) to be ineffective as a mating disruptant. When released at 600 mg/ha/h, mating of tethered virgin females and captures of males at EEOH-baited traps were reduced by only 40% and 50% respectively.

2.7. SUMMARY

Whilst consensus has still to be reached on the role of vision in mate location, pheromone appears to be of critical importance in the mediation of all but perhaps close-range behaviours. The manipulation of those behaviours to achieve mating disruption by the release of relatively large quantities of synthetic pheromone should be possible. Indeed the sedentary nature and

restricted host range of codling moth are characteristics of a species amenable to control by such a method.

However the variable results obtained in mating disruption trials in terms of levels of trap catch suppression and of fruit damage suggest that current mating disruption materials and/or techniques may be too poorly understood for the method to be considered a reliable alternative to insecticides for control of codling moth.

In seeking to develop a more effective system, account should be taken of Roelofs (1978) Threshold Hypothesis, an implication of which is that the more closely a synthetic pheromone blend resembles its natural counterpart, the more efficacious it will be as a mating disruptant. As behavioural activity has been demonstrated for two of the minor components of the codling moth pheromone gland, it is a reasonable assumption, based on Roelof's hypothesis, that their inclusion in the pheromone blend should make a more effective mating disruptant. An evaluation of multi-component blends and of pheromone-related compounds as mating disruptants follows.

3. RESEARCH

3.1 EVALUATION OF SINGLE AND MULTI-COMPONENT BLENDS

Introduction.

In trials preceding those described in this thesis, Rothschild and Vickers (1982) released EEOH from either one or two rubber tubing dispensers/tree in 100-tree plots to measure the effect of release rate on suppression of catch at synthetic pheromone and virgin female-baited traps. They found the relationship between the level of suppression and release rate to be inconsistent. On some occasions rates of 2-3 mg/ha/h were sufficient to suppress catch by 90% at both bait types whilst at other times a much higher release rate was required to achieve similar results. In a subsequent trial in which 'high' and 'low' insect density orchards (based on larval infestation levels) were treated with EEOH there was some evidence that in the low density site there was a threshold release rate of *ca* 10 mg/ha/h, above which the reduction in trap catch exceeded 90%. No such threshold was evident in the 'high' density site, where release rates of 20-40 mg/ha/h reduced catches by only 50-75% and in which fruit damage had reached 22% by early December. Insecticide applications at the 'low density' site precluded assessments of fruit damage.

The identification of behaviourally-active components other than EEOH (Arn *et al* 1985; Einhorn *et al* 1986; Bartell *et al* 1988) and the implications of Roelofs' (1978) Threshold Hypothesis (previously discussed) provided the rationale for conducting the following research:

1. field trials to compare multi-component blends and EEOH alone in terms of their attractiveness to males;

2. small plot trials to measure the influence of pheromone composition, release rate and dispenser distribution on trap catch suppression;

3. whole-orchard trials to determine the ability of multi-component blends to disrupt mating so that fruit damage was restricted to a commercially-acceptable level;

4. Observations of male behaviour :

1. in the field in air permeated with either EEOH alone or a 3-component blend, where the ability of males to orientate towards and land at or near pheromone baits was compared;

2. in the field in air permeated with EEOH, where male activity levels were measured;

3. in the laboratory, where the original bio-assay (Bartell *et al* 1988) indicating that 12OH and 14OH had significant behavioural activity was repeated.

Note: Full descriptions of traps, food lures, pheromone purity, dispensers and baits and the orchards used in the trials are provided in the Appendices.

3.1.1 Attractiveness to males.

Bartell *et al* (1988) found that baits containing a 1:0.5:0.1 ratio of EEOH:12OH:14OH were no more attractive to codling moth in the field than to EEOH alone, but provided no details of the pheromone concentrations they used. According to Roelofs' (1978) Threshold Hypothesis, the inferiority of an 'off' blend (ie, one without some components, or an incorrect component ratio) may be overcome by releasing larger quantities than would otherwise be required of the correct blend to achieve maximum catch. It is possible, therefore, that the baits containing only EEOH were releasing sufficient pheromone to mask any differences that might have existed between the single and 3-component blends.

There is no evidence to suggest that EEOH alone is inferior to the blend released by virgin females (Batiste *et al* 1973(a), Mantey *et al*, quoted in Maitlen *et al* 1976). Again, the quantities of synthetic EEOH

released in these trials (loadings of 1 mg were used by Batiste *et al;* those used by Mantey *et al* were not stated) may have been sufficient to compensate for the possible absence of other behaviourally-active components. It is also possible that the females did not release pheromone for the entire period during which males were active, thus contributing to a reduction in trap catch compared with those baited with synthetic pheromone

Trials were thus conducted with 1-500 µg concentrations of EEOH, both in single and multi-component blends, in an attempt to demonstrate whether the addition of minor components to EEOH enhanced trap catch. In one of the trials catches were compared with those obtained with virgin females.

Materials and methods.

Pheromone concentrations and component ratios. On three separate occasions EEOH concentrations of 500 µg (Trial 1), 50 µg (Trial 2) and 10 µg and 1 µg (Trial 3) were released from rubber tubing for the purpose of comparing the attractiveness of EEOH alone with a 1:0.5:0.1 ratio of EEOH:12OH:14OH ('3-component' blend) and a 1:0.5:0.1:0.1 ratio of EEOH:12OH:14OH:E9-12OH ('4-component' blend). E9-12OH was included in one of the blends because of its substantial presence in both the

gland (5.3%) and effluvia (6.2%), Arn *et al* (1985), although it had no demonstrated behavioural activity.

In Trials 1 and 2 delta traps were hung side by side ca 1.6 m above ground level on trees in Stevenson's orchard in five groups of 4, each group being separated by at least 12 m and each containing a representative of the 4 treatments (EEOH alone, 3-component, 4-component and a blank). By presenting the treatments in this fashion males approaching a group of traps were able to make a 'choice' between plumes.

The blank trap was included as a means of validating the method, as substantial catches in this trap would suggest that any differences there may have been between pheromone blends had been masked. All traps were aligned in the same direction relative to the prevailing north-westerly winds. The 500 µg trial was conducted over 3 days (4.11-6.11.86) and the 50 µg trial over 31 days (7.11-8.12.86). In the latter trial the baits were replaced on days 7, 17 and 24.

Trial 3 was conducted in Georgeovitch's orchard between 20.12.91-10.1.92, in which 10 and 1 µg concentrations of EEOH alone were compared with similar concentrations in 3 and 4-component blends. Virgin females were also evaluated.

All baits were placed in delta traps and hung on the

edge of the canopy at *ca* 1.6 m. The 5 replicates of each treatment were placed in 3 rows of 9 traps and 1 row of 8 traps, the traps being 7.5 m apart within the rows and 10 m apart between rows. Virgin female traps were baited with two 3-5 day old females housed in plastic cylinders (2.5 cm diameter, 7 cm long) enclosed at both ends with copper gauze and suspended above the sticky surface of a delta trap.

All baits were replaced weekly and the females at least weekly, but more often where they had died. Traps were cleared twice weekly over the period of the trial.

Results.

There were no significant differences between the 1, 3 or 4-component blends at either the 500 µg, 50 µg, 10 µg or 1 µg EEOH concentrations (Table 2). Catches at the blank traps in Trial 2 were proportionately higher than those at the blank traps in Trial 1. As the former trial was conducted over a much longer period (3 days vs. 31 days), it is possible that the vegetation and traps themselves had become contaminated with pheromone, making it difficult for males to distinguish between sources in close proximity to one another.

Regardless of the reason, the fact that the blank traps had caught substantial numbers of moths indicated that placement of traps side by side was not an appropriate

means of determining the relative attractiveness of pheromone blends. For this reason treatments were separated in Trial 3. Previous trapping data (Rothschild and Vickers, unpublished) had indicated that in this situation blank traps caught virtually nothing and they were not included in the trial.

Again, there were no significant differences between the mean catch at 1, 3 or 4-component blends at a given EEOH concentration, or between virgin female trap catch and any of the 1 µg loadings of synthetic pheromone. (Table 2).

EEOH release rates from the rubber tubing dispensers were not determined, but losses of 1 mg loadings from rubber tubing determined in a subsequent trial (Section 3.3.1), were found to be 114 ng/h. Assuming that release rates are proportional to loadings, as was the case for losses of Z8-12Ac from rubber tubing (T.E Bellas, personal communication), the rates will have been approximately 57.0, 5.7, 1.1 and 0.1 ng/h for 500, 50, 10 and 1 µg loadings respectively. The latter rate, 0.1 ng/h, is similar to that determined for virgin females. (0.13 ng/h., Section 3.4).

No.	EEOH (µg)	No. of components 1	3	4	Virgin female	Blank
1	500	11.0 a	9.8 a	7.6 a	-	0.8 b
2	50	24.6 a	20.4 a	21.6 a	-	5.0 b
3	10	4.8 a	6.4 a	2.4 a	-	-
	1	3.2 a	2.4 a	1.8 a	2.6 a	-

Table 2. Comparison of mean catch at traps baited with EEOH, 3 and 4-component blends and virgin females. Means within a given trial not followed by the same letter are significantly different at $p < 0.05$ (Duncan's MRT).

3.1.2 Factors affecting suppression of trap catch.

A preliminary step in the assessment of potential mating disruptants is to determine whether they are able to suppress catches at pheromone traps. If they have little effect in this regard then it is most unlikely that they will significantly influence levels of mating in the wild population.

The capacity of EEOH to suppress trap catch has previously been demonstrated (Rothschild and Vickers 1982). The purpose of the following trials was to determine how various pheromone blends (containing significant proportions of EEOH), dispenser placement

and pheromone release rate influenced the level of suppression.

In the first trial one 3 and one 4-component blend and three dispenser densities (1, 2 and 4 dispensers/tree) were evaluated in terms of their ability to suppress trap catch.

Effect of pheromone blend on trap catch suppression.

Materials and methods.

Treatments. Fourteen 25-tree (5x5) plots separated from each other by at least 20 m were set up in Spurway's orchard. Six of the plots were treated with a 3-component blend of EEOH:12OH:14OH in a 1.0:0.5:0.1 ratio - two with 1 Shin-Etsu (S-E) dispenser/tree, two with 2/tree and two with 4/tree. Another six were treated with a 4-component blend of EEOH:12OH:14OH:E9-12OH in the ratio 1.0:0.5:0.1:0.1 - two with 1 dispenser/tree, two with 2/tree and two with 4/tree. The remaining two plots were left untreated as controls. The trial was conducted between 25.9.86-30.1.87.

Pheromone traps. Each plot was monitored with five delta pheromone traps baited with 1 mg of the 4-component mix described above. The baits and traps were changed on days 48 and 82 of the 127-day trial. Catches were recorded and removed *ca* weekly.

Pheromone loss rates. Because the trial was conducted in an orchard some distance from the laboratory, it was necessary to seek the co-operation of the orchardist to perform some of the routine tasks, such as clearing the traps, changing baits and determining loss rates.

The latter task was simplified by having the orchardist remove, wrap in aluminium foil and store in a domestic freezer two 3-component and two 4-component dispensers each week from a sample of 30 dispensers of each blend whose initial pheromone column length had previously been determined. At the end of the trial pheromone losses for each weekly sample were plotted, from which loss rates were calculated.

Insecticide applications. Azinphos methyl was applied on four occasions during the trial at *ca* 1kg active ingredient (a.i.)/ha.

Results.

Pheromone trap catch. Mean captures/trap in all pheromone-treated plots were significantly lower than those in the controls ($p<0.001$), but were not significantly different from one another (Table 3). The the 3-component blend was as effective in suppressing catch as the 4-component.

The high mean catch in the plot treated with 4 dispensers/tree of the 3-component blend was largely due to a catch of 16 moths in one trap situated on the upwind border of the treated area. Mean catch in the remaining traps in this treatment was 0.9.

Treatment		Mean catch
No. comp.	Disp./tree	(n = 10)
3	1	1.6 a
	2	0.2 a
	4	2.4 a
4	1	3.0 a
	2	2.7 a
	4	0.3 a
control	-	37.0 b

Table 3. Effect of dispenser density and blend on suppression of catch at pheromone traps. Means not followed by the same letter are significantly different at $p<0.001$. (Duncan's MRT).

Pheromone loss rates. The mean loss rate with 4 dispensers/tree was 21.8 mg/h/h and the range 8.9 - 55.0

mg/ha/h. (There was no significant difference between loss rates of dispensers with either 3 or 4 components).

Effect of dispenser distribution on trap catch.

In the previous trial there was some suggestion that the level of trap catch suppression might be influenced by the number of dispensers/tree, although differences between treatments were not significant. However even if catches in plots treated with 4 dispensers/tree had been significantly lower than in those with 1/tree for example, the trial design would not have allowed a distinction to have been made between pheromone release rate and the number of dispensing points/tree. Both factors will have an influence on pheromone distribution throughout the treated area and hence the probability of males encountering pheromone.

The following two trials were designed to determine the effect of varying dispenser distribution (ie number of points within the treated area from which pheromone was released) whilst maintaining the same pheromone release rate throughout the different treatments. In the first trial the experimental units were individual trees, whilst in the second 25-tree plots were used.

Trial 1. Materials and methods:

Treatments. Thirty-two 20 mm lengths of rubber tubing were loaded with 1 mg EEOH and evenly distributed around the circumference of individual apple trees at a mean height of *ca* 1.5 m in Banks' orchard in various combinations of release points and sources/release point (Table 4).

Each treatment was replicated 5 times. Five untreated trees served as the control. The treatments were arranged as a Latin square and were separated from one another by a minimum of 24 m. Loss rates were not determined.

Pheromone traps. A delta trap baited with 1 mg of EEOH was placed in each tree at *ca* 1.5 m and cleared of its catch on 9 occasions during the 40-day trial conducted between 21.12.88 - 30.1.89. Traps were placed at least 1 m from the nearest pheromone release point.

Results:

Mean captures in all pheromone-treated trees were significantly different from the control catch but not from one another (Table 4).

Treatment		Mean catch
Release points/tree	Sources/ release point	/trap
2	16	3.2 a
4	8	1.6 a
8	4	1.4 a
16	2	3.4 a
control	-	15.2 b

Table 4. The influence of release point number on trap catch. Means followed by a different letter are significantly different at $p<0.001$.(Duncan's MRT)

Trial 2. Materials and methods.

Treatments. Eight 25-tree (5x5) plots separated from each other by at least 20 m were set up in Spurway's orchard. Two of the plots were left untreated as controls and the remaining six each treated with 48 S-E dispensers containing a 1.0:0.5:0.1 blend of EEOH:12OH:14OH, so that there were either three dispensers in each of 16 trees, four in 12 trees or eight in 6 trees (Appendix 6.2). Thus pheromone release rate in each plot at any given time was similar, but its distribution was varied. There were two replicates of

each treatment and the trial was conducted between 30.1-26.3.87.

Pheromone traps. Each plot was monitored with 5 delta pheromone traps baited with 1 mg of the 3-component blend described above. All baits were changed on day 27 of the 55-day trial and catches were recorded and removed weekly. Trap positions are shown in Appendix 6.2.

Pheromone loss rates. The method used to determine loss rates was as described in the trial to determine the effect of pheromone blend on trap catch suppression. (Section 3.1.2.).

Results.

Trap catch. Mean trap catches are shown in Table 5.

Pheromone loss rates. Mean loss rate for the period of the trial was 24.3 mg/ha/h and the range 2.5 - 77.5 mg/ha/h. Loss rates exceeded 20 mg/ha/h during the first 25 days (during which period 62% of the total pheromone trap catch was recorded) but did not exceed 5 mg/ha/h during the second 25-day period.

Treatment		Mean catch
Dispensers/ treated tree	Treated trees/ 25-tree plot	
3	16	6.6 a
4	12	4.3 a
8	6	19.4 b
control	-	29.4 b

Table 5. The influence of release point number on trap catch. Means followed by a different letter are significantly different at $p<0.001$. (Duncan's MRT)

Discussion

Rothschild (1975) found that there was no significant difference between levels of trap catch suppression of the oriental fruit moth (*Cydia molesta*) when pheromone release points were placed at rates varying from 1/50 - 1/400 m^2 and with a constant release rate per unit area. However there was a significant reduction in the level of suppression with 1 point/800 m^2 and the author thought it possible that at this rate disruptive levels of pheromone were localized, enabling males to locate pheromone traps.

In mating disruption trials to determine the effect of release rate and distance between release points on

catches of the rice stem borer (*Chilo suppressalis*) at synthetic baits, Tatsuki (1990) found that providing the release rate exceeded 2.1 mg/ha/h, catch was suppressed by 90% or more with release points spaced up to 16 m apart (1/256 m^2). At spacings of 32 m (1/1024 m^2) and a release rate of 2.5 mg/ha/h catches were suppressed by 42%, but the level was restored to 89% by increasing the release rate to 9.8 mg/ha/h. When dispensers were placed 64 m apart, catch was still suppressed by 73% at a release rate of 9.8 mg/ha/h and Tatsuki suggested that even greater spacings could be used providing the release rate was high enough.

Because of the dispenser placement patterns used in the present trials it is difficult to equate the results with those obtained by other workers. However if each tree containing pheromone dispensers is considered to be a single dispensing point and the mean distance between dispensing points is calculated (eg 3 dispensers on each of 16 trees within the 25-tree plots = 1 dispensing point/37.2 m^2), then a significant decrease in the level of suppression occured once the area covered by each dispensing point exceeded 50 m^2. These results contrast with those of Charmillot (1990), who reported that satisfactory results were obtained in mating disruption trials of codling moth where dispensers were applied at a rate of 100/ha (1/100 m^2) in orchards of 1-3 ha, and 40-50/ha (1/200-250 m^2) in larger orchards.

Distribution of dispensing points within the same tree had no effect on the level of trap catch suppression (Trial 1). Although release rates were not determined in this trial, on the basis of those established for 1 mg loadings of EEOH in a subsequent trial (Section 3.3.2), they are estimated to have been *ca* 114 ng/h. There were 32 dispensers/tree, equating to a release rate of 1.0 mg/ha/h, which is substantially less than those obtained in Trial 2. Taking the results of both trials into account, it would appear that providing the distribution of pheromone is adequate, considerable reductions in catch at traps baited with synthetic pheromone can be achieved with very low pheromone release rates.

3.1.3 Mating disruption studies.

The scale of the trials designed to investigate factors influencing trap catch suppression was too small to allow assessment of treatment effects on the incidence of mating in wild females and levels of fruit damage. Inclusion of these parameters in the assessment required that immigrating mated females be excluded from the treated areas.

There are several means of achieving this, including the treatment of areas large enough to minimise the effects of immigration, treatment of entire isolated orchards and erection of physical barriers to prevent immigration.

The first experiment in this series of trials measured the effects of a multi-component blend on catch at pheromone traps and food lures, and the incidence of mating and age distribution in females caught at food lures.

Trial 1. Materials and methods.

Treatments. The northern end of Wood's orchard was divided into three adjacent blocks, two of 240 trees (10x24) trees and a smaller one of 216 trees (9x24) separating them. The two larger blocks were treated with either one or two S-E dispensers/tree placed *ca* 1.8 m

above ground level on the tree circumference and diametrically opposite each other in the plot with two/tree. The central block was left untreated as a control. The long sides of the blocks were approximately parallel to the prevailing wind direction (north-westerly) which was, however, very variable. The trial was conducted between 17.10.84 - 28.3.85.

Dispensers and loss rates. The dispensers were loaded with a 1:1:0.1 ratio of EEOH:12OH:14OH, a ratio derived from an analysis of gland contents (Bartell *et al* 1988). Pheromone loss rates were determined weekly by measuring the length of the pheromone column remaining ('meniscus recession') on a sample of 10 dispensers. The dispensers were not replaced during the trial.

Pheromone traps and food lures. Each block was monitored with 5 delta pheromone traps, 5 food lures and 20 trap bands. The pheromone baits (1 mg of the same component mix used in the disruption dispensers) were replaced once a month and the traps every two months. Both the traps and the food lures were read and cleared weekly, the catch from the latter being stored in 70% alcohol for later dissection to determine mating status.

Physiological age of the females was also determined, based on the method of Nel (1940), in which the number of eggs remaining in the ovarioles and the extent to

which the fat bodies had been depleted were scored. Age 1 represented the youngest and age 3 the eldest females.

The purpose of assessing the age distribution of females caught in the food lures was to determine whether mating had been delayed by the pheromone treatments, which, it was assumed, should be reflected by an increase in the proportion of mated females in category 3.

Assessments of fruit damage. Although the intention was to assess levels of fruit damage throughout the trial, commercial pressures dictated that the orchardist protect his crop by applying insecticide, thus masking any effect the treatments may have had on the incidence of fruit damage. For this reason no assessments were made.

Insecticide applications. The orchard was treated with azinphos methyl (*ca* 1kg a.i./ha applied with a tractor-drawn mist sprayer) for codling moth control on 6 occasions during the trial.

Results.

Pheromone trap catch. Catches are summarised in Table 6. Mean captures/trap in the two pheromone treatments were significantly lower than those of the control but were not significantly different from each other. Over the period of the trial (153 days) the level of trap

suppression was 74% and 69% in the one and two dispensers/tree treatments respectively. (Percent suppression = (control catch - treated catch / control catch) x 100))

A plot of pheromone trap catches in all plots (Fig 1) revealed four distinct peaks. A comparison of the levels of trap suppression obtained during these periods (Table 6) suggest that the effects of the treatments were greatest during the first period (42 days), but suppression of trap catch remained above 60% for most of the remaining 111 days.

Food lure catch. Catches are summarized in Table 7. The treatments had no significant effect on the proportion of females that had mated or on their age at capture. The proportions of females that had mated more than once were 5.4%, 7.3% and 2.5% in the 1 dispenser/tree, 2 dispensers/tree and control plots respectively.

Pheromone loss rates. Pheromone release rates are given in Table 8.

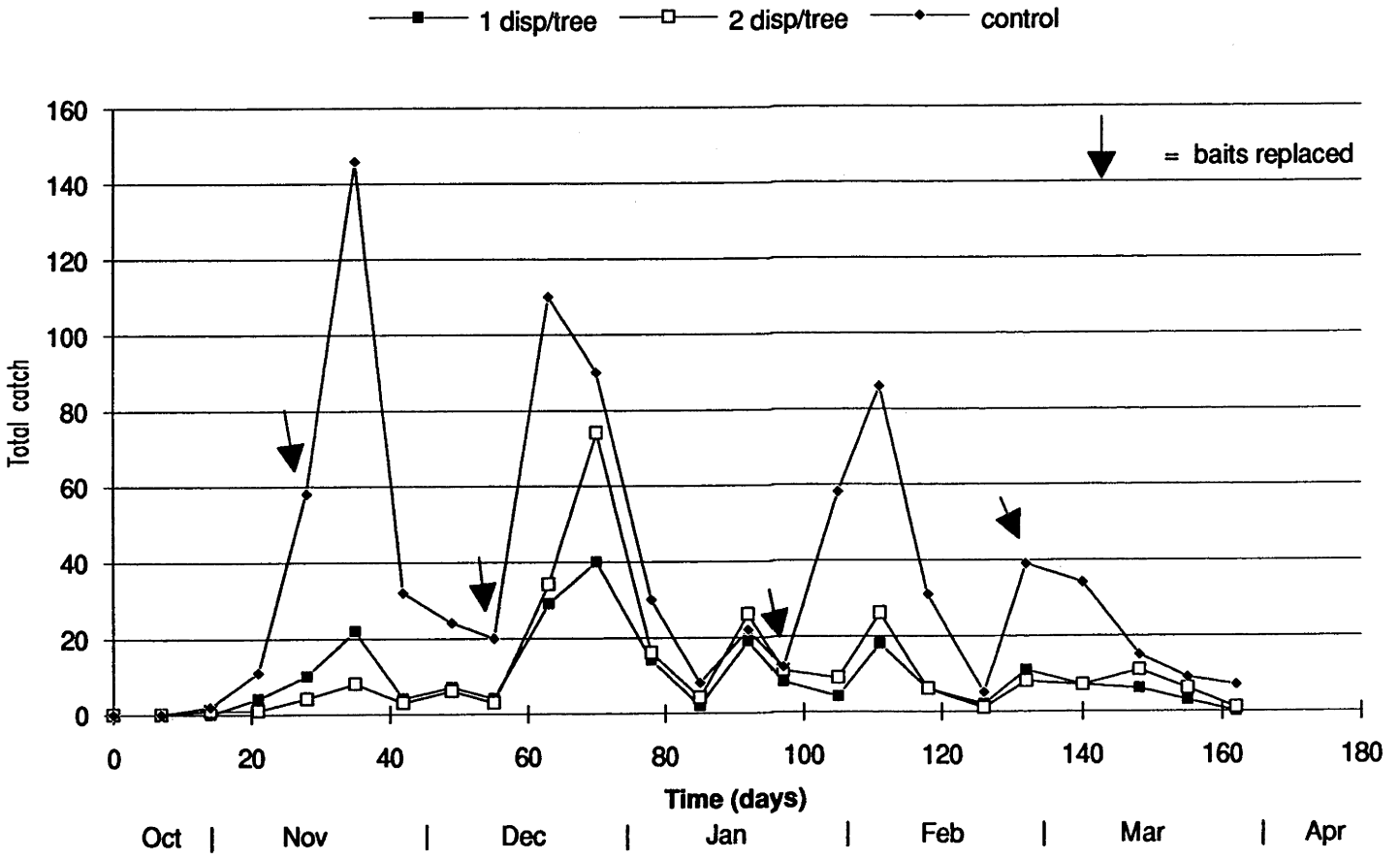

Fig. 1. Total pheromone trap catch in treated and control plots, indicating dates that pheromone baits were replaced.

Period (days)	Treatment (disp/tree)	Mean catch/trap (n=5)	% suppression
1- 42	control	56.6 a**	
	1/t	9.4 b	83.4
	2/t	4.6 b	91.8
43- 77	control	51.6 a*	
	1/t	17.8 b	65.5
	2/t	26.2 b	49.2
78-117	control	42.8 a***	
	1/t	11.4 b	73.4
	2/t	15.8 b	63.1
118-153	control	20.8 a**	
	1/t	5.4 b	74.0
	2/t	6.6 b	68.3
TOTAL	control	171.8 a***	
	1/t	44.0 b	74.4
	2/t	53.2 b	69.0

Table 6. Catch at pheromone traps and %trap suppression. Mean catches not followed by the same letter within a given period are significantly different at the following levels: * = $0.01 < p<0.05$, ** = $0.005 < p < 0.01$, *** = $p < 0.001$. (Duncan's MRT).

Treatment	Total catch		% females	Age (%)		
	m	f	mated	I	2	3
1 disp/tree	8	49	90	67	27	6
2 disp/tree	15	63	88	66	24	7
control	15	56	98	76	13	11

Table 7. Total catch, % females mated and physiological age of adults caught at food lures.

Period (days)	Mean loss mg/ha/h (±SD)
1-42	4.8 (0.2)
43-77	5.8 (0.1)
78-117	5.5 (0.1)
118-153	2.1 (0.1)

Table 8. Mean pheromone release rates over successive periods in mating disruption trial.

Discussion.

Although trap catches in the pheromone-treated plots were significantly lower than those in the control plot, the number and proportion of mated females recovered from the food lures did not differ significantly between treatments. This might be explained by the treated plots

being too small to negate the effects of immigrating mated females from the adjacent control plot.

As codling moth has only 3 generations a year, at least one of the peaks indicated in Fig. 1 is an artifact. There appears to be some correspondence between the peaks and bait replacement, suggesting that old baits were much less attractive than fresh ones.

The next two trials involved treatment of the entire (isolated) orchard. On both occasions the grower agreed to withhold insecticide applications unless periodic assessments of fruit damage indicated that they were needed.

The disadvantage in treating the entire orchard was that there could be no control plot with which results from the treated orchard could be compared. However this was more than off-set by being able to determine the incidence of fruit damage, mating in wild females and numbers of immature stages without the threat of immigrating mated females confounding the results.

Trial 2. Materials and methods.

Treatment. Each tree was treated with four S-E dispensers containing a 1:1:0.1 ratio of EEOH:12OH:14OH. Two were tied to twine laid over the tops of the tree canopy and the other two were placed on opposite sides

of the tree at *ca* 1.5 m above ground level. This dispenser placement was adopted in an attempt to ensure that pheromone was evenly distributed throughout the canopy. Four additional dispensers were placed in each tree in similar positions to the first placement 14 weeks after the trial began. Loss of pheromone was determined by meniscus recession at weekly intervals for the first four weeks of the trial and thereafter at *ca* monthly intervals.

Pheromone traps. The orchard was divided into 10 plots of approximately equal tree numbers. In the centre of each plot a trapping station consisting of an aluminium pole was placed, from which 2 delta traps were positioned so that they hung at the edges of the upper and lower thirds of the canopy (mean heights 1.5 m and 2.5 m respectively.

The traps were baited with a 1 mg loading of EEOH:12OH:14OH in a 1:1:0.1 ratio. Catches were recorded and removed weekly and baits and traps were changed *ca* every 4 and 8 weeks respectively. The trial was conducted between 26.9.85-15.4.86.

Food lures. On a tree adjacent to those with pheromone trap stations a food lure was hung *ca* 1.5 m above ground level. Catches were removed weekly and stored in 70% ethanol for subsequent sexing and to determine the mating status and physiological age of females. The

brown sugar/terpinyl acetate solution was topped up as required.

Numbers of immature stages. Each trap station tree was banded with 75 mm-wide corrugated cardboard, trapping larvae moving towards the base of the tree to pupate or overwinter. The bands provided information about the movement of 5th instar larvae throughout the season and about larval populations at the end of the trial. A 600 mm length of corrugated cardboard was placed around the butts of the trees and the remainder (1800 mm) around the base of major limbs. The first bands were put up when the trial began and were thereafter replaced at *ca* monthly intervals.

Larvae recovered from the bands were sexed by examining them for the presence of gonads on the dorsal surface (indicating males), and pupae by counting the number of freely-articulated ventral abdominal segments (3 in females, 4 in males).

Assessment of fruit damage. At approximately monthly intervals beginning early in January and continuing until April, 6000 fruit chosen at random (100 per tree on 6 trees in the vicinity of each of the 10 trapping stations) were examined for evidence of codling moth attack. Only 'deep entries' (as opposed to superficial 'stings') were scored and no account was taken of windfalls.

Insecticide applications. The orchardist was instructed to apply insecticide for codling moth control if he considered that it was warranted by the levels of fruit damage revealed by the surveys.

Results.

Pheromone trap catch. A total of 419 moths were caught in the pheromone traps in the course of the trial, of which 404 (96.4%) were taken in the upper traps. As in the previous trial, a noticeable feature of the catch pattern was that in the week following each bait replacement there was a substantial rise in the number of moths caught (Fig 2).

Food lure catch. Catches of both sexes at food lures and the mating status and age of females are summarised in Table 9. Of the 89 females caught, 86 (92%) had mated. Despite the absence of a control plot, it was obvious that the treatment had no effect on the incidence of mating.

Pheromone loss rates. Pheromone loss rates are shown in Table 10.

Immature stages. Larvae began to appear in the trap bands between days 79-104 (15 December - 9 January) and in substantial numbers between days 105-132 (January 10

- February 7) and 133-174 (February 8 - March 20) (Table 11). A mean of 15.8 larvae was recovered from each band.

Assessment of fruit damage. Fruit damage is summarized in Table 12.

Period (days)	Catch m	Catch f	% f. with indicated no. spermatophores 0	1	2	% f. of age 1	2	3
1- 60	27	29	15	81	4	30	55	15
61-120	14	60	5	90	5	22	43	35
Total	41	89	8	87	5	24	47	29

Table 9. Catch at food lures showing mating status and physiological age of females.

Period (days)	Placement	Loss rate (mg/ha/h) Mean (± sd)	Range
1-105	1st	16.8(0.85)	2.0-25.2
106-204	1st	16.7(0.85)	8.8-25.4
	2nd	27.8(1.18)	15.1-38.7

Table 10. Pheromone loss rates from field-exposed Shin-Etsu dispensers.

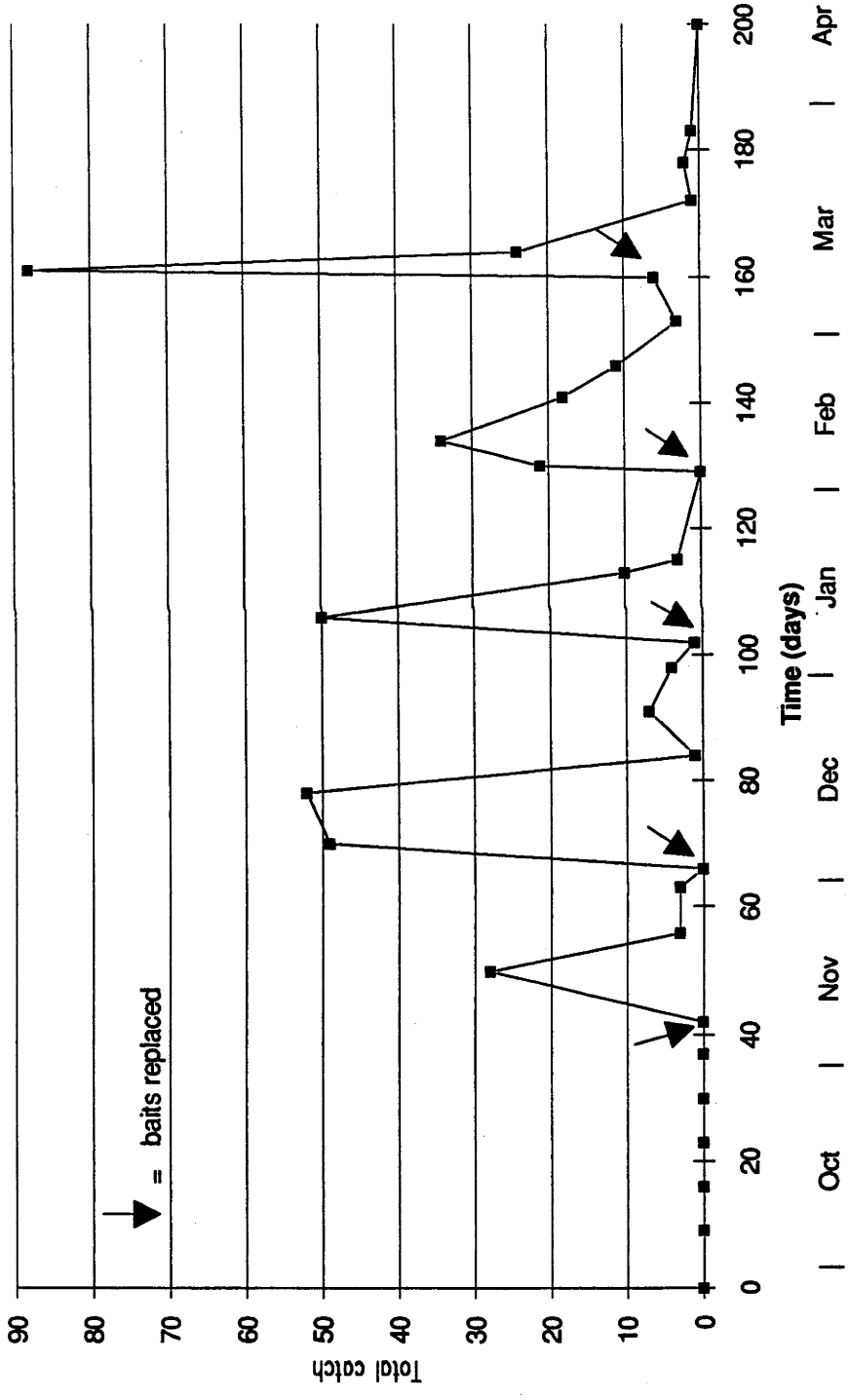

Fig. 2. Total pheromone trap catch in pheromone-treated orchard, indicating dates that pheromone baits were replaced.

Period (days)	Larvae m	Larvae f	Larvae ?	Pupae m	Pupae f	Pupae ?	Pupal cases	Total
1- 41	0	0	0	0	0	0	0	0
42- 78	0	0	0	0	0	0	0	0
79-104	2	1	0	0	0	0	5	8
105-132	9	16	12	0	1	0	29	67
133-174	49	66	3	0	0	9	78	205
Total	60	83	15	0	1	9	112	280

Table 11. Total number of immature stages recovered from trap bands placed around 10 trees.

	Trap site										
Day	A	B	C	D	E	F	G	H	I	J	Total	%
78	0	16	1	0	0	6	7	1	4	9	44	0.7
105	0	44	4	1	12	11	17	7	14	22	132	2.2
133	2	67	14	7	37	42	27	22	72	24	314	5.2
174	1	66	4	6	31	46	39	22	84	34	333	5.6

Table 12. Number of damaged fruit in the vicinity of the 10 trapping stations. 600 fruit sampled/site/occasion. Stations A, C and D were in pears.

Insecticide applications. Azinphos methyl (*ca* 1kg a.i./ha applied with a tractor-drawn mist sprayer) was applied for codling moth control on days 95 and 137.

Discussion.

The treatment failed to disrupt mating. Substantial numbers of males were caught at pheromone traps, a high proportion (92%) of females caught in the food lures had mated, fruit damage was unacceptably high (particularly considering that azinphos methyl had been applied on two occasions) and many immature stages were recovered from the trap bands, all of which indicated that the treatment had little effect on mating levels.

Pheromone release rates, apart from those during the first week of the trial (2.0 mg/ha/h), were similar to those reported by Charmillot (1990) (10-40 mg/ha/h) in his successful codling moth mating disruption trials and were well above those considered adequate for mating disruption of other species (eg the oriental fruit moth *Cydia molesta*, 6 mg/ha/h, Rothschild 1975; Ponderosa pine tip moth *Rhyacionia zozana*, *ca* 5.2 mg/ha/h, Niwa *et al* 1988). (As a multi-component blend was used in the present trials, the quantities of EEOH released would have been less than indicated by the overall release rates).

The effect of fresh baits on trap catch, first noticed

in Trial 1, was even more evident in Trial 2. This phenomenon was further investigated in Section 3.3.

Analyses of pheromone glands taken from field-collected females (Whittle, unpublished data) indicated that the 12OH content was approximately half that previously quantified in females from the laboratory culture (Bartell *et al* 1988). For this reason all subsequent trials involving combinations of EEOH:12OH:14OH were conducted using a 1:0.5:0.1 ratio.

A fourth component, (*E*)-9-dodecadiene-1-ol (E9-12OH) was added. This component had not previously been included in the disruptant trials because it had no known behavioural function. However failure of the 3-component blend led to a re-evaluation of potential disruptant components and the monoene was included on the basis of its significant presence in the gland (5.3%) and effluvium (6.2%) (Arn *et al* 1985).

Trial 3. *Materials and methods.*

Treatment. Every tree in Wood's orchard was treated on 25.9.86 (before the first codling moth emergence) with four S-E dispensers containing a 1.0:0.5:0.1:0.1 ratio of EEOH:12OH:14OH:E9-12OH. Dispensers were placed in each quadrant of the tree on the circumference, two at the top of the canopy (*ca* 3.0 m) and two mid-canopy (*ca* 1.5 m).

On day 126 of the 183-day trial (25.9.86 - 26.3.87) a further series of four dispensers was placed in each tree in positions corresponding with those of the first series.

Pheromone loss rates. The lengths of pheromone columns in 30 (1st placement) and 20 (2nd placement) dispensers was measured before exposure in the field. At weekly intervals 2 dispensers were removed, wrapped in aluminium foil and placed in a domestic freezer. At the end of the trial the amount of pheromone that had been lost from each dispenser was determined by meniscus recession and from a plot of cumulative loss, loss rates were calculated.

Pheromone traps. Five delta traps, evenly spaced throughout the orchard, were placed on internal trees at mid-canopy level and at least 1 m from the nearest disruption dispenser. They were baited with 1 mg of the 4-component mix used as the disruptant. When additional pheromone dispensers were put in (day 126), another five pheromone traps were installed on the boundaries - two on the eastern side and one each on the other sides. The purpose of these traps was to indicate whether aerial concentrations of pheromone were reduced on upwind boundaries by wind movements. If they were then one would expect a reduced level of trap catch suppression compared with traps within the orchard. Traps were read

and cleared weekly and baits and traps changed monthly and every 2 months respectively.

Food lures. A food lure was placed in a tree adjacent to each of the five internal pheromone traps. Catches were removed weekly and stored in 70% ethanol for subsequent examination to determine sex ratio and the mated status and physiological age of the females. The solution was replenished weekly.

Assessments of fruit damage. Damage was assessed on two occasions - day 126 and near harvest (day 183.) Seventy five fruit on each of the 10 pheromone trap trees (plus an adjacent tree if there were insufficient fruit) were examined for deep entries only. Windfalls were not examined. Two of the trees sampled were Packham pears, an early-maturing variety which had been harvested before the second survey took place and for which only the orchardist's estimate of damage was available.

Results.

Pheromone trap catch. Pheromone trap catch is shown in Table 13. The first male was caught on day 57 (21 November) and there was no significant difference between the mean numbers of males caught in the internal and boundary traps. (Although the prevailing wind was north-westerly, it was very variable and for this reason comparisons were made between numbers of moths caught at

boundary traps and numbers caught at internal traps, rather than between catches at the prevailing upwind edge and the other edges).

Period (days)	Mean catch/trap (SD)	
	Internal (n=5)	Boundary (n=5)
1-126	2.8 (2.6)	-
127-183	5.4 (6.8)	9.4 (8.1)

Table 13. Mean pheromone trap catch/trap at internal and boundary traps. Means over days 127-183 were not significantly different. (Students T-test).

Food lure catch. Catches at the food lures are summarised in Table 14. The proportion of females that had mated during the first (days 1-126) and second periods (days 127-183) were, respectively, 89% and 96%. A much larger proportion of the females caught during the second period were classified as age 3, and multiple-mating, which had not been detected during the 1st period, was relatively common during the second period.

Assessment of fruit damage. The level of fruit damage increased substantially between the two surveys and was considerably heavier at harvest on the boundaries,

(21.7%), than internally (9.0%) (Table 15). Damage levels on the two pear trees harvested before the second survey were estimated by the orchardist to be 50%. As damage in the apples (all sites) averaged 15.3% this is probably an over-estimate, given that in the previous year on the same orchard pear damage at harvest averaged 3.6% compared with 46% in apples (Trial 2).

Period (days)	Catch		% f. with indicated no. of spermatophores			% f. aged		
	m	f	0	1	>1	1	2	3
1-126	56	96	12	89	0	41	38	20
127-183	13	25	4	72	24	14	22	64
Total	69	121	11	84	5	35	35	29

Table 14. Catch at food lures with data on mating status and physiological age of females.

Pheromone loss rates. Eight dispensers from the 30 (first placement) designated to be removed at weekly intervals to determine loss rates were misplaced. Consequently losses for days 89-126 could not be determined. With this exception, mean loss for days 1-126 was 11.5 mg/ha/h (range 2.5-22.5) and 21.1 mg/ha/h (2.9-61.6) for days 127-183. (Actual values for the latter would have been slightly higher than stated as no

account was taken of pheromone still being released from the first placement).

Day	Mean % deep entries		
	Internal	Boundary	All trees
126	1.6	1.6	1.6
183	9.0	21.7	15.3

Table 15. Summary of fruit damage (deep entries only) in apples.

Insecticide applications. Azinphos methyl (*ca* 1kg a.i./ha applied with a tractor-drawn mist sprayer) was applied for codling moth control on days 81, 103 and 139.

Trial 4. Mating disruption in large field cages. Materials and methods.

Treatments. Six 9-tree (3x3) plots at least 25 m apart were selected in Stevenson's orchard. A single delta trap was placed at a height of *ca* 1.8 m in the central tree of each plot and, on the basis of total catch over an 11-day period preceding petal fall, (8.10-18.10.87) treatments were allocated as shown in Table 16.

To prevent movement of mated females into the pheromone-treated plots, each one was enclosed after pollination had been completed (ie at petal fall) by a knitted shadecloth cage which allowed 50% light transmission and which was supported by a metal tubing frame-work (Fig. 3). The cages measured 16.4 x 16.0 x 2.5-3.5 m, height depending on the slope of the ground.

Four Bio-dispensers were evenly spaced around the circumference of each tree in the treated plots, *ca* 1.8 m above ground level. Pheromone loss rates were determined by weekly measurements of meniscus recession on a sample of five dispensers.

The trial ran for 55 days (28.10-22.12.87), after which all pheromone dispensers were removed from the cages. Catches in the plots over the following 20 days provided further information about the influence of the treatment on the incidence of mating.

Pheromone traps. A single live trap baited with 1 mg of the 3-component blend used in the disruption dispensers was placed in the central tree of each plot and cleared daily except on week-ends. Live traps (Appendix 6.4) rather than tanglefoot-coated traps were used so that males could be sampled non-destructively and returned to the population. The baits were changed *ca* monthly.

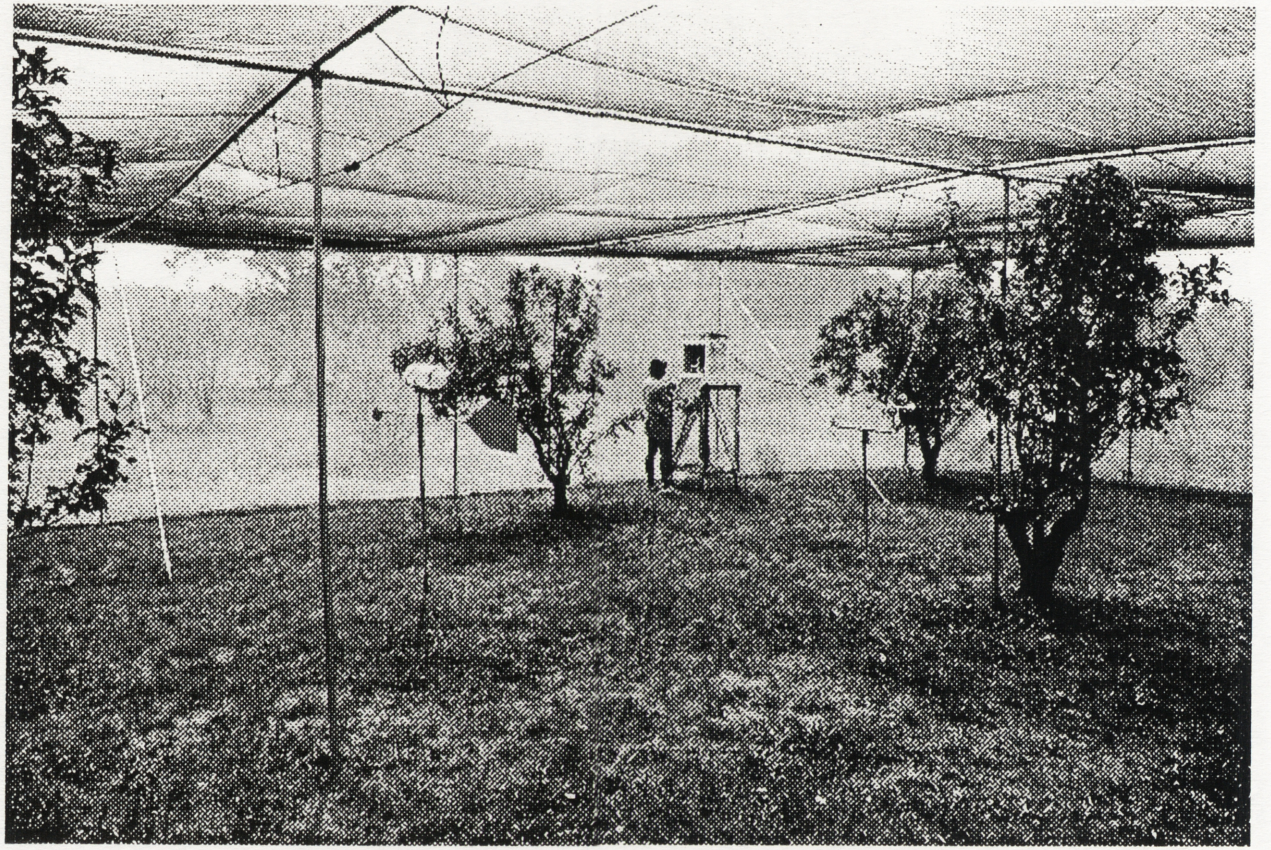

Figure 3. View from inside one of the large field cages used in Trial 4.

Number and sex ratio of wall captures and incidence of mating in females. On 24 occasions during the day and on four at dusk the internal and external walls of all the cages were searched for adults. Where possible (some were out of reach) these were captured, sexed and, with the exception of one or two females/cage/occasion, returned to the population. The retained females were dissected and their mating status determined.

Assessment of fruit damage. As part of the assessment procedure it was intended that 200 fruit selected at random in each plot be scored for codling moth damage. However a casual survey in mid-December revealed that in all plots, treated and control, damage levels exceeded 10% and the trial was terminated soon after.

Results.

Pheromone trap catch. Catches within individual cages (plots) are shown in Table 17. The mean daily total catch in the three pheromone-treated plots whilst the treatments were in place was 0.52 and in the control plots 2.35, representing a 78% reduction in trap catch. The means were significantly different at $0.001<p<0.005$ (Student's T-test). Catches over the 20 days following removal of the treatments are shown in Table 16.

Treatment	Plot	Pre-treatment	During treatment	Post-treatment
Pheromone	2	9	11	29
	4	14	1	41
	6	24	3	18
Control	1	25	48	38
	3	17	13	37
	5	9	12	40

Table 16. Total pheromone trap catches in pheromone-treated and control plots before pheromone treatments were in place, during treatment and after pheromone was removed.

Number and sex ratio of wall captures and incidence of mating in females.

Internal walls. Fewer adults were caught in the treated plots than in the controls (Table 17), both during the day and in the evening. Of those captured during the day, the sex ratio favoured females in the treated plots (1 male : 1.5 females), but was almost equal in the controls (1 male : 0.9 females). There was no sex ratio difference between treated and control (1 male : 0.6 females) during the evening.

There was no significant difference between the proportions of mated females in the treated (66.7%, n = 69) and control plots (74.7%, n = 71).

Period	n	Treatment	Catch				Ratio
			m	f	?	total	m:f
Day	23	pheromone	46	69	50	165	1:1.5
		control	95	83	111	289	1:0.9
Evening	4	pheromone	57	37	62	156	1:0.6
		control	70	43	103	216	1:0.6

Table 17. Number and sex ratio of adults captured on the internal walls of the cages.

External walls. More than twice as many adults were counted on the external walls of the control cages than were counted on those of the treated cages during the day. During the evening the difference was almost 3-fold. The trend was similar for both sexes, suggesting that both males and females were avoiding areas of high pheromone concentration. Males were predominant in both treatments, during the day as well as the evening (Table 18). The mating status of females caught on the external walls was not determined.

Pheromone loss rates. Mean loss rate was 174.2 mg/ha/h and the range 96.8 - 278.4 mg/ha/h..

Period	n	Treatment	Catch				Ratio
			m	f	?	total	m:f
Day	23	pheromone	11	4	10	25	1:0.4
		control	17	10	29	56	1:0.6
Evening	4	pheromone	4	1	2	7	1:0.3
		control	11	2	7	20	1:0.2

Table 18. Number and sex ratio of adults captured on the external walls of the cages.

Discussion.

In none of the trials was there any evidence to suggest that the incidence of mating had been affected by the pheromone treatments. Although Trial 1 was the only one in which a control plot was incorporated, it was clear from all trials that the treatments were ineffective as mating disruptants.

It is possible that population densities contributed to the failure to disrupt mating. In an unpublished report of mating disruption trials conducted in Switzerland between 1979-85, Mani (personal communication, 1986)

concluded that for mating disruption to succeed the larval population should be as low as possible and certainly less than 1000/ha. (Charmillot (1990) described a trial in which there were 120 diapausing larvae/tree in the year preceding the imposition of a mating disruption treatment. In each of the following 3 years, during which 71 mg of pheromone was applied annually, the larval population decreased about one fifth. However as there were no control plots for comparison, it is not possible to attribute the decline to the effects of the treatments).

At the end of the 1985-86 season (Trial 2) a mean of 15.8 larvae was recovered from each banded tree, (equivalent to over 4200 larvae/ha), most of which would have gone into diapause. Even allowing for *ca* 45% pupal mortality (Wearing 1979), Mani's threshold of 1000 larvae/ha would have been exceeded at the beginning of the 1986-87 season, which may in part explain the failure of the treatments to disrupt mating during Trial 3.

There is circumstantial evidence to suggest that mating had been delayed, if not prevented, by the treatments. In the first trial almost 76% of the females caught in the control plot were classified as being Age 1. Although this was similar to the proportions classified as Age 1 in the two pheromone treated plots (66% and 67%), because of the small size of the plots and their

proximity to one another there is likely to have been movement of females between treatments.

Thus there is some justification for pooling the catches of all three plots in this trial, in which case a mean of 70% is obtained for females of Age 1, compared with only 24% and 35% in the trials conducted in the following two years, where the entire orchard was treated with pheromone. The implication of these results is that mating had been delayed by the pheromone treatments.

3.1.4. Observations of behaviour.

Introduction.

In conjunction with field trials to determine whether multi-component blends were superior to EEOH alone in terms of their attractiveness as baits and, when released as a background, their ability to disrupt males seeking synthetic sources of pheromone, field trials were established to:

1. observe and compare the behaviour of males exposed to background levels of either EEOH, a 3-component blend or to 'pheromone-free' air (control) as they flew within the vicinity of a synthetic pheromone bait. (Pheromone-free air refers to air into which no synthetic

pheromone, apart from that released by the baits, has deliberately been released).

2. Observe and compare the behaviour of males confined in either air permeated with EEOH or to pheromone-free air in an attempt to determine whether the pheromone suppressed activity levels.

Evidence that components other than EEOH had a behavioural effect on codling moth males was produced by Bartell & Bellas (1981) using a bioassay apparatus colloquially known as the 'wheel'. At that time the identity of the components had not been determined, but subsequent chemical analyses of pheromone gland extract fractions and bioassays, again using the 'wheel' to confirm behavioural activity, indicated that they were 12OH and 14OH (Bartell *et al* 1988).

As previously stated, it was this finding that prompted the research described in this thesis. However failure of blends incorporating all three components to disrupt mating or indeed to have any effect on male behaviour in the field that was significantly different from that achieved with EEOH alone led to a repeat of the original assays purporting to show enhanced male sexual activity to the three-component blend. Attempts were also made to study male sexual behaviour in a wind tunnel.

The laboratory studies thus consisted of:

1. behavioural trials that compared the effects of EEOH and multi-component blends on male behaviour in order to verify the findings of Bartell *et al* (1988) that had indicated a behavioural role for 12OH and 14OH when presented together with EEOH. ('Wheel' studies);

2. attempts to elicit similar information from males exposed to various pheromone combinations and concentrations in a wind tunnel.

3.1.4.1. Field trials in air permeated with pheromone.

Trial 1. Behaviour of males exposed to either no pheromone (control), EEOH alone or a 3-component blend.

Materials and methods.

Treatments. Six 9-tree (3x3) plots separated by at least 40 m were established in Stevenson's orchard. Two were treated with EEOH, two with a 1.0:0.5:0.1 blend of EEOH:12OH:14OH and two were left untreated as controls.

Pheromone was released from S-E dispensers, four of which were equally spaced around the circumference of each treated tree at *ca* 1.5 m. The dispensers were

tagged, enabling them to be readily located and removed. The treatments were rotated twice during the trial so that every plot received each treatment on two occasions. As the observations were made over short periods (1-1.5 hours at a time), it was not possible to determine pheromone release rates using the standard method of measuring meniscus recession. For this reason temperatures were recorded during the observation periods and release rates estimated on the basis of rates previously established in controlled temperature rooms.

Immediately beside the central tree in each plot a 1m x 1m x 1m metal frame (hereafter termed a 'cube') was erected so that a limb from each tree projected into the frame to a point at or near its centre. (Fig. 4). From a central point, defined by intersecting diagonal rods, a bait loaded with 1 mg of the 3-component blend described above was suspended. The trial was conducted during the period 28.10 - 22.12.87.

Observations. An observer, stationed beside each frame, recorded moth behaviour within the cube for a period beginning about an hour before sunset to half an hour after, by which time light levels were too low to permit further observations. All moths that flew within the frame boundaries were recorded as having either:

1. flown straight through the cube or landed on the frame or foliage but not within 10 cm of the bait.

2. Landed on or within 10 cm of the bait and exhibited wing buzzing.

Although 10 cm was an arbitrary value, moths reaching this point were considered to have 'penetrated' the barrier imposed by the pheromone treatments and would most likely have mated had a calling female been the source of pheromone. Observations were made on 8 occasions over an 18-day period.

Two null hypotheses were established:

1. that there were no significant differences between treatments (including the controls) in terms of the total number of insects entering the cube;

2. that there were no significant differences between treatments (including the controls) in terms of the numbers that landed on or within 10 cm of the bait and wing buzzed.

Netting of adults. On several observation nights a second person netted adults once they had left the cube.

These catches were sexed and females dissected to determine their mating status.

Results.

Observations. The counts of moths in each of the 2 categories were analysed using a generalized linear model with Poisson error and log link to obtain an analysis of deviance (McCullagh & Nelder, 1989).

Both null hypotheses can be rejected. After adjusting for differences due to observation date (site differences were not significant), total numbers entering the cube and numbers landing on or within 10 cm of the bait were significantly higher in the control plots than in either of the two pheromone-treated plots. However differences between the two pheromone treatments were not significant. (Table 19).

Netting of adults. Of the 20 adults netted over four consecutive nights as they left the control cube, one was female. From the single and 3-component cubes respectively, two of the 14 and one of the 6 adults netted were female. Three of the 4 females caught in total had mated.

Treatment	n.	Mean response (±SE)	
		All flights	Landing
Control	10	43.9 a (2.1)	7.9 a (0.9)
1 component	9	10.0 b (1.1)	0.2 b (0.2)
3 component	9	7.4 b (0.9)	0.3 b (0.2)

Table 19. Adjusted means for moths entering the cubes and landing at or within 10 cm of the bait. Means within a response followed by the same letter are not significantly different at $p<.001$ (analysis of deviance).

Pheromone loss rates. Pheromone loss rates were estimated to range between 14-35 mg/ha/h in both the 1 and 3-component treated plots.

Trial 2. Materials and methods.

This trial was conducted within the large field cages described in Section 3.1.3, Trial 4 during the period 19.11-19.12.87.

Treatment. On four evenings at dusk, when males were sexually active, behavioural observations were made of males confined to mesh cone-shaped cages (45 cm diameter at the base, 40 cm high; Fig. 5).

Two 2-3 day old laboratory-reared males were placed in each of the cages, one of which was suspended in the central tree of each pheromone-treated (n = 3) and control plot (n = 3).

Observations. Observations were made at 10 min intervals for one min at a time over *ca* one hour, during which the time each male spent in either a sedentary or an active state (walking/wing buzzing or flying) was noted.

Pheromone release rates. Pheromone release rates were determined by meniscus recession as described in Section 3.1.3, Trial 4.

Analysis. The data were analysed using a generalised linear model with scaled binomial error to obtain an analysis of deviance. The influence of date, occasion on a given date, observer bias, field cage bias and treatment were determined.

Results.

Because activity levels in both the treated and control cages were very low on two of the observation nights, the proportion p = VA/VT (where VA = time spent active and VT = total observation period) was modelled on the logit scale so that the treatment had a smaller effect

on p when it was closer to 0 than it would when closer to 0.5.

The level of activity in the treated cages was approximately 16% that of the controls. This difference was highly significant ($F_{1, 25} = 29.93$, $p < 0.001$) and the results were consistent over both occasion ($F_{3, 25} = 0.71$, n.s.) and observer ($F_{8,25} = 1.34$, n.s.). The cages had significant but small effects ($F_{4, 25} = 3.63$, $p = 0.018$).

Pheromone release rate. Pheromone was released at a mean of 190 mg/ha/h (range 101-250 mg/ha/h).

Discussion.

In Trial 1 significantly fewer males entered the cubes treated with pheromone than entered the control cubes, for which there are several possible explanations:

1. males were avoiding areas of high pheromone concentration;
2. false trails created by the treatments diverted males from the trail created by the bait;
3. the trail created by the bait was indistinguishable in a 'fog' of pheromone created by the treatments;
4. male activity was suppressed by the high concentrations of pheromone.

With the exception of (1), these possibilities are covered by the various mechanisms proposed as means by which mating disruption might be achieved, recently reviewed by Carde (1990).

The results from Trial 2 suggest that suppression of male activity may have been at least partly responsible for fewer males having entered the treated cubes in Trial 1. However it should be noted that the effect in Trial 2 was achieved with the pheromone release rates of 101-250 mg/ha/h, considerably greater than those of Trial 1 (14-35 mg/ha/h).

Charmillot and Bloesch (1987) demonstrated an increase in trap catch in pheromone-permeated orchards as the concentration of pheromone in the baits was increased. This suggests that the treatment had raised the threshold above which males would respond to pheromone, and that only from baits charged with the higher concentrations was the threshold exceeded. In both of the present trials the pheromone treatments may have raised the threshold required to elicit responses in at least some of the male population and could account for fewer numbers having entered the cubes in Trial 1 and lower levels of activity in Trial 2.

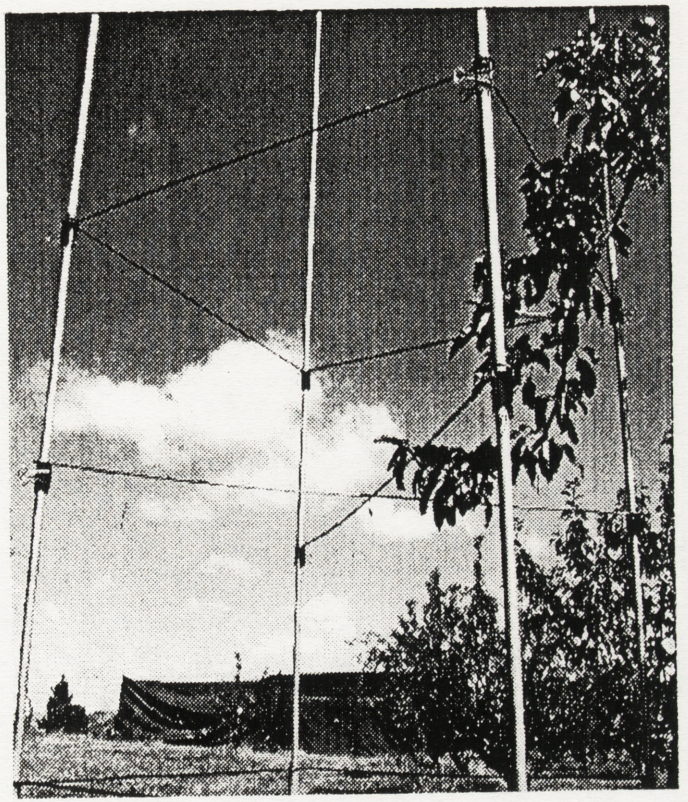

Fig. 4. View of cube used in Trial 1. The pheromone bait was attached to the intersecting diagonal rods.

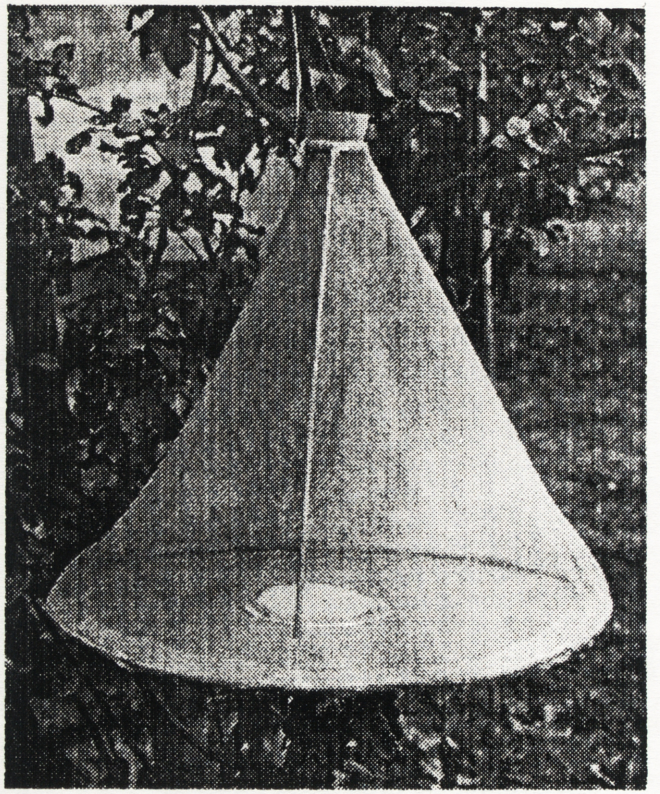

Fig. 5. View of one of the cages in which males were confined during observations of their behaviour (Trial 2).

3.1.4.2. Laboratory bioassays.

1. Wheel studies.

Materials and methods.

Bioassay apparatus. The bioassay apparatus used was the same as that described by Bartell and Shorey (1969). In brief it consisted of 12 chambers arranged around the rim of a horizontal wheel and in which the males were placed. Air was delivered to the chambers from below and exhausted through the top and pheromone was introduced by placing the source in the air stream beneath the chambers. Males in only one chamber were exposed to pheromone at a given time.

The only significant change made to the apparatus was to place the males directly into the glass chambers of the apparatus rather than firstly into cylindrical copper-wire mesh cages, thus providing a clearer view of the insects. Copper mesh bases and tops prevented males from escaping.

Insects. All bioassays were conducted with 2 to 5-day old virgin males from the laboratory culture that were only used once and then discarded.

Pheromone sources. The synthetic sources were of the same concentrations used by Bartell *et al* (1988), ie EEOH: 3.6×10^{-10} g, 12OH: 3.6×10^{-10} g, 14OH: 0.4×10^{-10} g, except for Trial 4, in which concentrations of all components were increased ten-fold.

Female gland extracts were prepared by T.E.Bellas in the manner described in the account of the original bioassay (Bartell and Bellas 1981).

All components were dissolved in cyclohexane and dispensed in aliquots of 10 µl onto 10 mm diameter glass filter paper discs. Fresh discs were prepared for each assay.

Bioassays. Bioassays were conducted in a fashion similar to that used by Bartell and Bellas (1981) and more fully described in Bartell and Shorey (1969), with the following differences:

1. only two males were placed in each chamber. This allowed individuals to be more readily observed than would have been possible with 5 males/chamber and reduced the likelihood of the results being biased by one male's behaviour influencing that of another/others;

2. the identity of the sources being assayed were unknown to either the observer or the recorder at the time of presentation, thus eliminating the possibility

of any inadvertent bias in interpretation or recording of the behaviour;

3. controls (filter paper loaded with 10 µl of the solvent cyclohexane) were included in each set of bioassays.

Males reared under a 14:10 L:D regime were placed in the chambers 30 minutes before the onset of scotophase (ie dark period) and allowed to acclimate to ambient conditions. Light intensity was gradually reduced to an incident illumination of 3.5 lx over this period. In the early trials air was delivered from a remote compressor to each unit at 1.2 l/min. and was turned on 30 minutes before the measurements began. However when Trial 1 was repeated, the responses obtained from males exposed to the controls were not significantly different from those exposed to pheromone. A further series of bioassays indicated that contaminants in the compressed air were accumulating in the bio-assay apparatus and stimulating the males, and behaviour similar to that observed in response to pheromone was induced simply by disturbing the air flow. Subsequent trials were therefore conducted with 'instrument grade' bottled air delivering 1.2 l/min. to each unit, but because of the limited life of this source, air was turned on one minute before measurements began.

The males in each chamber were observed for 120 seconds. At intervals of 30, 15 and 0 seconds before and 15, 30, 45, 60, 75 and 90 seconds after the stimulus was introduced the number of males walking and wing buzzing/flying were scored, counts taken before introduction of the stimulus representing the base level of activity. At time 0 the stimulus was inserted for 10 seconds and then removed.

Bioassay schedule. The bioassay schedule is shown in Table 20.

Analyses. For each chamber, two variables were defined for analysis:

1. the number of insects wing buzzing/flying;

2. the number of insects walking or wing buzzing/flying.

Each of these variables is binomial, with only three possible values, 0, 1 or 2. The appropriate form of analysis for such data is an analysis of deviance, obtained by fitting a generalized linear model with binomial error and logit link (McCullagh and Nelder 1989).

Assay	Stimuli	n	Comments
1 & 2	EEOH	30	compressor air
	EEOH+12OH	30	
	EEOH+14OH	30	
	EEOH+12OH+14OH	30	
	control	30	
3	EEOH	20	bottled air
	EEOH+12OH	20	
	EEOH+14OH	20	
	EEOH+12OH+14OH	20	
	control	20	
4	EEOH	30	x 10 conc., bottled
	EEOH+12OH	30	air
	EEOH+14OH	30	
	EEOH+12OH+14OH	30	
	control	30	
5	fe 2	6	fe = female extract
	fe 1+2	6	Nos. refer to 1st
	fe 2+3	8	2nd & 3rd fractions
	fe 1+2+3	7	bottled air
	control	6	
6	fe 2	8	bottled air
	EEOH	8	
	control	8	

Table 20. Schedule of treatments used in 'wheel' bioassays.

From these analyses it was apparent that the data could be more concisely summarised by considering three groups of observation times; pre-stimulus (-30, -15, 0), post-stimulus 1 (15, 30, 45) and post-stimulus 2 (60, 75,

90). Analyses of deviance were then run on these groups, with responses now being the number of occasions out of 6 that the behaviour of interest (ie 'walking' or 'walking and wing buzzing/flying') was observed.

Differences across times for each bioassay were examined by performing analyses of deviance of each of the variables including time as an extra factor. This time effect was further split into three components; pre- vs. post-stimulus observations, between pre-stimulus observations and between post-stimulus observations. The analysis also included interactions between treatments and each of the three time components.

Results.

Analyses of deviance. Deviances were obtained and significance was tested for the following factors:

run: differences due to the occasion (day) on which a set of observations was made.

stimulus: differences between stimuli over all nine observations.

chamber: differences between chambers.

prepost: differences over all stimuli between, before and after the stimulus was applied.

pretime: differences over all stimuli between observations at -30, -15 and 0 seconds.

posttime: differences between observations at 15, 30, 45, 60, 75 and 90 seconds.

stimulus.prepost: differences between relative activity levels for the 5 stimuli before and after the stimulus was applied.

stimulus.pretime: differences between relative activity levels for the 5 stimuli at -30, -15 and 0 seconds.

stimulus.posttime: differences between relative activity levels for the 5 stimuli at 15,30,45,60,75 and 90 seconds.

Results of the analyses of deviance for the proportion of insects wing buzzing/flying are given in Table 21 and for the proportion walking or wing buzzing/flying in Table 22.

Highly significant differences were obtained between pre-stimulus and post-stimulus levels of activity (refer to large analyses of deviance, Tables 21 and 22), so detailed presentation of the results concentrates on an examination of the three time groups (Tables 23 and 24).

Claims that 12OH and 14OH stimulated male sexual activity when presented together with EEOH in the 'wheel' bioassay (Bartell *et al* 1988) were not substantiated when the bioassay was repeated. Only on one occasion did the addition of both saturated alcohols lead to a significantly higher level of activity (wing

buzzing/flying and walking and wing buzzing/flying) than was achieved with EEOH alone. (Tables 23 and 24, Assay 1, Post-stimulus 1).

Whilst there were significant differences between treatments on some occasions, no one treatment was consistently better (or worse) than another. Activity levels in the blanks were sometimes similar to those induced by the pheromone treatments, although in Assay 2 (Tables 23 and 24) this was attributed to contamination of the apparatus by unidentified compounds from the air compressor. (Described in materials and methods).

Attempts to duplicate the earlier results of Bartell and Bellas (1981), which had indicated the presence of behaviourally-active fractions in the female pheromone gland other than natural EEOH (Fraction 2), were also unsuccessful. Whilst the number of repetitions in this trial (Assay 5) were small (6), there was no indication that any combination of the fractions tested was superior to Fraction 2 in terms of the levels of sexual activity they induced.

The problems encountered with contaminants in the compressed air could not have been recognised by Bartell and Bellas (1981) or Bartell *et al* (1988) because they had no controls. Indeed there may not have been a contamination problem. However as all the equipment used in the current trials was the same as that used in the

earlier bioassays (with the exception of the minor changes indicated in materials and methods), contamination seems the most likely explanation for the differences between the results of the bioassays of Bartell *et al* and those reported here.

Factor	Ass. 1 d.f.	dev.		Ass. 2 d.f.	dev.		Ass. 3 d.f.	dev.	
run	18	151.1	***	15	204.7	***	7	41.2	***
stimulus	4	112.2	***	4	5.7	n.s.	4	54.1	***
chamber	141	509.9	***	130	342.2	***	84	343.9	***
prepost	1	255.8	***	1	541.0	***	1	81.9	***
pretime	2	31.5	***	2	13.0	**	2	1.8	ns
posttime	5	123.0	***	5	109.3	***	5	52.4	***
stim.prepost	4	24.0	***	4	7.0	n.s.	4	20.5	***
stim.pretime	8	3.9	ns	8	9.8	n.s.	8	9.2	ns
stim.posttime	20	18.5	ns	20	17.8	n.s.	20	26.2	ns
Residual	1272	929.5		1160	1010.9		728	501.8	
TOTAL	1475	2159.4		1349	2261.4		863	1132.7	

Factor	Ass. 4 d.f.	dev.		Ass. 5 d.f.	dev.		Ass. 6 d.f.	dev.	
run	12	119.9	***	2	13.9	***	1	13.3	***
stimulus	4	80.2	***	4	15.7	**	2	33.1	***
chamber	133	485.7	***	26	85.0	***	20	77.6	***
prepost	1	423.1	***	1	13.4	***	1	18.4	***
pretime	2	2.6	n.s.	2	1.7	ns	2	4.4	ns
posttime	5	91.0	***	5	15.2	***	5	24.4	***
stim.prepost	4	13.8	**	4	8.4	ns	2	8.4	*
stim.pretime	8	12.6	n.s.	8	7.9	ns	4	3.4	ns
stim.posttime	20	15.0	n.s.	20	22.8	ns	10	19.4	*
Residual	1160	858.1		224	98.3		168	103.5	
TOTAL	1349	2102.0		296	282.3		215	305.9	

Table 21. Analyses of deviance for proportion of insects wing buzzing/flying. The level of significance is indicated thus: *** = p <0.001; ** = p<0.01; * = p<0.05.

	Ass. 1			Ass. 2			Ass. 3		
Factor	d.f.	dev.		d.f.	dev.		d.f.	dev.	
run	18	135.4	***	15	165.9	***	7	37.4	***
stimulus	4	67.8	***	4	6.3	ns	4	37.4	***
chamber	141	466.4	***	130	290.4	***	84	325.3	***
prepost	1	191.3	***	1	379.9	***	1	60.6	***
pretime	2	21.8	***	2	9.0	*	2	0.8	ns
posttime	5	117.8	***	5	100.9	***	5	51.0	***
stim.prepost	4	18.7	***	4	16.0	**	4	9.9	*
stim.pretime	8	4.3	ns	8	5.1	ns	8	5.2	ns
stim.posttime	20	10.6	ns	20	13.9	ns	20	24.1	ns
Residual	1272	1179.5		1160	1256.6		728	682.0	
TOTAL	1475	2213.6		1349	2243.9		863	1233.6	

	Ass. 4			Ass. 5			Ass. 6		
Factor	d.f.	dev.		d.f.	dev.		d.f.	dev.	
run	12	143.3	***	2	5.4	ns	1	22.2	***
stimulus	4	57.6	***	4	26.6	***	2	29.7	***
chamber	133	415.4	***	26	101.1	***	20	87.2	***
prepost	1	210.7	***	1	5.1	*	1	3.8	ns
pretime	2	8.1	*	2	0.3	ns	2	2.5	ns
posttime	5	103.1	***	5	21.8	***	5	17.3	**
stim.prepost	4	16.8	**	4	7.7	ns	2	5.0	ns
stim.pretime	8	11.5	ns	8	7.3	ns	4	2.6	ns
stim.posttime	20	14.2	ns	20	20.3	ns	10	27.5	**
Residual	1160	1151.7		224	163.7		168	138.8	
TOTAL	1349	2132.4		296	359.3		215	336.4	

Table 22. Analyses of deviance for proportion of insects walking or wing buzzing/flying. The level of significance is indicated thus: *** = $p < 0.001$; ** = $p < 0.01$; * = $p < 0.05$.

Assay	Treatment	Pre-stimulus (-30, -15, 0)	Post-stimulus I (15, 30, 45)		Post-stimulus II (60, 75, 90)	
1	Blank	0.08 a	0.12 c	***	0.09 c	***
	EE	0.05 a	0.38 b		0.27 ab	
	EE+12	0.10 a	0.39 b		0.24 b	
	EE+14	0.10 a	0.51 a		0.33 a	
	EE+12+14	0.11 a	0.48 a		0.29 ab	
2	Blank	0.09 a	0.48 a		0.34 a	
	EE	0.05 a	0.48 a		0.33 a	
	EE+12	0.07 a	0.52 a		0.45 a	
	EE+14	0.09 a	0.53 a		0.36 a	
	EE+12+14	0.05 a	0.54 a		0.38 a	

Table 23. Mean proportion of insects wing buzzing/flying. Means not followed by the same letter within a given bioassay and column are significantly different. Significances were obtained from single degrees of freedom contrasts between the 5 treatments within the analyses of deviance. * = $p<.05$; ** = $p<.01$; *** = $p<.001$

Assay	Treatment	Pre-stimulus (-30, -15, 0)		Post-stimulus I (15, 30, 45)		Post-stimulus II (60, 75, 90)	
3	Blank	0.04 b	*	0.17 c	***	0.16 b	**
	EE	0.15 a		0.21 bc		0.14 b	
	EE+12	0.12 a		0.50 a		0.32 a	
	EE+14	0.14 a		0.42 a		0.30 a	
	EE+12+14	0.11 a		0.29 b		0.23 ab	
4	Blank	0.07 a		0.24 b	***	0.13 c	***
	EE	0.08 a		0.52 a		0.43 a	
	EE+12	0.08 a		0.51 a		0.38 ab	
	EE+14	0.08 a		0.51 a		0.31 b	
	EE+12+14	0.08 a		0.54 a		0.38 ab	

Table 23. (cont.) Mean proportion of insects wing buzzing/flying. Means not followed by the same letter within a given bioassay and column are significantly different. Significances were obtained from single degrees of freedom contrasts between the 5 treatments within the analyses of deviance. * = $p<.05$; ** = $p<.01$; *** = $p<.001$

Assay	Treatment	Pre-stimulus (-30, -15, 0)	Post-stimulus I (15, 30, 45)	Post-stimulus II (60, 75, 90)
5	Blank	0.05 a	0.11 a	0.00 b **
	f.e. 2	0.15 a	0.22 a	0.18 a
	f.e. 1+2	0.05 a	0.13 a	0.07 ab
	f.e. 2+3	0.04 a	0.30 a	0.19 a
	f.e. 1+2+3	0.02 a	0.13 a	0.12 ab
6	Blank	0.10 a	0.08 b ***	0.06 b **
	EE	0.15 a	0.44 a	0.31 a
	f.e. 2	0.15 a	0.58 a	0.25 a

Table 23. (cont). Mean proportion of insects wing buzzing/flying. Means not followed by the same letter within a given bioassay and column are significantly different. Significances were obtained from single degrees of freedom contrasts between the 5 treatments within the analyses of deviance. * = $p<.05$; ** = $p<.01$; *** = $p<.001$

Assay	Treatment	Pre-stimulus (-30, -15, 0)	Post-stimulus I (15, 30, 45)		Post-stimulus II (60, 75, 90)	
1	Blank	0.13 a	0.22 d	***	0.16 c	***
	EE	0.12 a	0.42 c		0.31 ab	
	EE+12	0.18 a	0.44 bc		0.28 b	
	EE+14	0.15 a	0.56 a		0.37 a	
	EE+12+14	0.17 a	0.50 ab		0.35 a	
2	Blank	0.19 a	0.52 a		0.39 a	
	EE	0.13 a	0.49 a		0.36 a	
	EE+12	0.12 a	0.58 a		0.49 a	
	EE+14	0.15 a	0.57 a		0.42 a	
	EE+12+14	0.13 a	0.55 a		0.41 a	

Table 24. Mean proportion of insects walking or wing buzzing/flying. Means not followed by the same letter within a given bioassay and column are significantly different. Significances were obtained from single degrees of freedom contrasts between the 5 treatments within the analyses of deviance. * = $p<.05$; ** = $p<.01$; *** = $p<.001$

Assay	Treatment	Pre-stimulus (-30, -15, 0)	Post-stimulus I (15, 30, 45)		Post-stimulus II (60, 75, 90)	
3	Blank	0.13 a	0.26 b	***	0.24 b	*
	EE	0.20 a	0.27 b		0.24 b	
	EE+12	0.24 a	0.53 a		0.38 a	
	EE+14	0.19 a	0.48 a		0.37 a	
	EE+12+14	0.15 a	0.34 b		0.27 ab	
4	Blank	0.19 a	0.34 b	***	0.18 c	***
	EE	0.19 a	0.59 a		0.47 a	
	EE+12	0.22 a	0.55 a		0.42 a	
	EE+14	0.17 a	0.55 a		0.33 b	
	EE+12+14	0.22 a	0.57 a		0.43 a	

Table 24. (cont). Mean proportion of insects walking or wing buzzing/flying. Means not followed by the same letter within a given bioassay and column are significantly different. Significances were obtained from single degrees of freedom contrasts between the 5 treatments within the analyses of deviance.* = $p<.05$; ** = $p<.01$; *** = $p<.001$

Assay	Treatment	Pre-stimulus (-30, -15, 0)	Post-stimulus I (15, 30, 45)	Post-stimulus II (60, 75, 90)
5	Blank	0.08 b *	0.14 a	0.00 c ***
	f.e. 2	0.31 a	0.27 a	0.29 a
	f.e. 1+2	0.14 b	0.22 a	0.10 bc
	f.e. 2+3	0.17 ab	0.38 a	0.30 a
	f.e. 1+2+3	0.07 b	0.25 a	0.19 ab
6	Blank	0.21 a	0.08 b **	0.23 a
	EE	0.35 a	0.58 a	0.38 a
	f.e. 2	0.30 a	0.60 a	0.31 a

Table 24. (cont). Mean proportion of insects walking or wing buzzing/flying. Means not followed by the same letter within a given bioassay and column are significantly different. Significances were obtained from single degrees of freedom contrasts between the 5 treatments within the analyses of deviance. * = $p<.05$; ** = $p<.01$; *** = $p<.001$

2. Wind-tunnel studies.

The wind-tunnel is a more suitable apparatus than the 'wheel' for studying male response to pheromone because it enables the entire sequence of behaviours elicited by pheromone stimuli to be observed - excitation, take-off, oriented flight, landing and attempted copulation with the source. Conversely the 'wheel' can only measure the intensity of the initial excitation (of which wing fanning is presumed to be indicative) and perhaps some element of flight. Unfortunately one cannot be sure that such behaviours in this context are necessarily sexual, particularly as in the current trials they also appeared to be elicited by non-pheromone contaminants in the air supply.

For these reasons attempts were made to compare male responses to EEOH, accumulated pheromone effluvia and multi-component blends in a wind tunnel. However despite introductions of field-collected codling moth to invigorate the laboratory culture from which males for bio-assay were drawn, numerous changes to pre- and within-assay conditions (light intensity and direction, temperature, humidity and wind speed) and the testing of a wide range of pheromone concentrations, it was not possible to develop a reliable bio-assay.

On most occasions individuals or pairs of males placed

in the pheromone plume would become excited and leave their cages, but then fly either to a side wall or the floor where they would usually remain for the duration of the test (5-10 min). Occasionally some would take off again and fly upwind to the bait.

Some success was obtained with males that were introduced to the tunnel *ca* 5 min before dusk and allowed to fly freely within it during the 1 h test period. On most occasions there were at least as many landings at the baits as there were moths in the tunnel, but because it was not possible to distinguish new landings from re-visits, meaningful data could not be gathered on the relative attractiveness of different baits. Furthermore it was quite likely that the behaviour of moths flown in 'bulk' was influenced by one another. On numerous occasions males that had 'locked on' to the plume aborted their flights when others approached to within a few cm or made physical contact.

Males that landed at the source when flown in 'bulk' were collected for subsequent testing as individuals, but their responses were no better than those achieved with 'non-selected' individuals and no further attempts were made to use the wind tunnel as a bioassay.

3.2 EVALUATION OF PHEROMONE-RELATED COMPOUNDS.

Introduction.

There are many examples amongst lepidoptera of chemicals, closely related in structure to their pheromone, that inhibit sexual attraction (eg Roelofs and Comeau 1971; Klun and Robinson 1972; Schwarz *et al* 1990).

Field and laboratory studies have identified several compounds, also known as 'anti-pheromones,' that suppress codling moth catches when released at or near the pheromone source. Roelofs *et al* (1972) found that the geometric isomers of EEOH were inhibitory, whilst George *et al* (1975) and Arn *et al* (1974) respectively, identified several ester and ether derivatives of EEOH and (*Z*)-8-dodecenyl acetate (Z8-12Ac) as inhibitors. Hathaway *et al* (1979) found the acetate equivalent of EEOH, (*E,E*)-8,10-dodecadien-1-ol acetate (EEAc) to be a potent inhibitor.

Rothschild (1974) urged caution in proclaiming compounds to be inhibitors on the basis of their ability to suppress trap catch, after finding that the distance between the pheromone source and the 'inhibitor' (dodecyl acetate, 12Ac) influenced catches of the oriental fruit moth, *Cydia molesta*. Whilst they were suppressed when 12Ac was released from a source within

the same trap as the pheromone, the effect was reversed and catches were higher in the treated plot than the control when it was released from sources several metres from the trap. Shorey (1977) indicated in his review of pest control with behaviour-modifying chemicals that compounds which suppressed trap catch if evaporated from a source immediately adjacent to the pheromone had little or no effect when released as a background treatment.

The issue of distance between pheromone and inhibitor was not investigated by Arn *et al* in relation to Z8-12Ac, but it was to some extent taken into account by Hathaway *et al* (1979) in their trials with EEAc. They found that hollow fibre formulations hand placed *ca* 15 cm from pheromone traps or broadcast from a ground rig or helicopter all disrupted trap catch by 100% for at least 1 week, after which time the effect began to diminish. No information about the distribution of broadcast formulations of EEAc was provided and it is not possible to determine the proportion of the sources located at distances greater than 15 cm from the traps.

As Z8-12Ac had not been evaluated as an inhibitor when evaporated from a source outside the trap, and was readily available dispensed from S-E dispensers used for mating disruption of oriental fruit moth, trials were undertaken to determine:

1. the effect of distance between the pheromone source and Z8-12Ac on catch at pheromone traps;

2. the ability of Z8-12Ac to suppress trap catch when released as a background treatment at rates well above those found adequate for suppression with pheromone components.

The promising results obtained by Hathaway *et al* (1985) with EEAc were further investigated, but with fewer release points and a higher release rate than they had used.

3.2.1. Suppression of trap catch.

1. The effect of distance between Z8-12Ac and the pheromone source.

Materials and methods.

each at least 12 m apart,

Delta pheromone traps were baited with 1 mg of a 1:1:0.1 blend of EEOH:12OH:14OH. A single 20 mm length of rubber tubing loaded with 10 mg of Z8-12Ac was placed beside the pheromone bait (0 mm), or 2 such sources were placed 150, 300, 600 or 1200 mm away on opposite sides of the open ends of the trap and in the same horizontal plane. (A source of Z8-12Ac was placed opposite each entrance to the trap so that males following the pheromone plume

towards the trap would be exposed to the compound regardless of wind direction). Traps baited with pheromone but with no Z8-12 Ac in the vicinity served as controls. There were 5 replicates of each treatment. The trial was conducted in Stevenson's orchard from 10.2.-11.3.86.

Results.

Trap catch. The effect of Z8-12 Ac diminished as distance between the source and pheromone increased. With a separation of 600 mm or more there was no significant difference between the treatment and the control. (Table 25).

Distance between pheromone and Z8-12 Ac (mm)	Mean catch /trap
0	0.0 a
150	0.8 a
300	2.2 ab
600	4.2 bc
1200	6.4 c
Control	7.0 c

Table 25. Effect of distance between the pheromone bait and Z8-12Ac on trap catch. Means not followed by the same letter are significantly different at $p < 0.001$. (Duncan's MRT).

Loss rates of Z8-12Ac. Loss rates of Z8-12 Ac from the rubber tubing dispensers were not determined in this trial, but on the basis of release rates measured in the laboratory they were estimated to approximate 22 ng/h. (T.E. Bellas, CSIRO Division of Entomology, personal communication 1991).

2. Background treatments of Z8-12 Ac and trap catch suppression.

Although the results of the trial described above were not encouraging in terms of Z8-12 Ac being an effective inhibitor, a trial was undertaken to evaluate the compound when released from sources that were generally at least 1.2 m from the pheromone, but which contained *ca* 350 times as much of the compound as the rubber tubing dispensers. The rationale for doing so was that by increasing the amount of active ingredient applied, increased aerial concentrations might compensate for the effects of distance between pheromone and inhibitor sources noted in the previous trial.

Materials and methods.

Treatments. Four groups of 5 S-E dispensers were applied to each tree in a 0.55 ha plot of apple trees at Spurway's orchard. Each dispenser contained *ca* 70 mg of a 93:7 blend of Z8-12 Ac:E8-12 Ac. Two groups were

placed in the top of the canopy (*ca* 3.5 m) on opposite sides of the tree and the other two at *ca* 1.6 m at the canopy edge, again on opposite sides of the tree. Loss of inhibitor was determined by meniscus recession at weekly intervals.

An untreated plot of 0.59 ha served as the control. Both this plot and the one in which Z8-12 Ac was applied were treated on 6 occasions with azinphos methyl at 1 kg a.i./ha during the course of the 26-week trial (10.10.85 - 17.4.86).

Pheromone traps. Five trapping stations, each with a delta pheromone trap at crown level (*ca* 3.5 m) and another at 1.5 m, were established in each plot. The traps were baited with 1 mg of a 1:1:0.1 blend of EEOH:12OH:14OH and the baits were changed *ca* monthly. Traps in the treated plot were at least 1 m from the Z8-12Ac sources and all traps were cleared weekly.

Results.

Pheromone trap catch There were no significant differences between catches in the treated and control plots at either the upper or lower traps (Table 26).

Loss rates. Z8-12Ac was lost at a mean rate of (range 64.7 - 102.9 mg/ha/h).

Z8-12Ac		Control	
Upper	Lower	Upper	Lower
41.2	12.4	54.4	8.8

Table 26. Mean catch/trap (n = 5) in Z8-12 Ac -treated and control plots. Means at a given height (upper, lower) were not significantly different from one another. (Student's t-test).

3.2.2. Suppression of trap catch with EEAc.

The major difference between this trial and those of Hathaway *et al* (1985) relates to distribution of the inhibitor. Hathaway *et al* implied that as both hand-applied (within 15 cm of pheromone baited traps) and broadcast formulations (distribution unknown) of EEAc released from Conrel fibres had significantly suppressed trap catch, it was not essential that the sources be placed in close proximity to the pheromone source for it to be effective.

Shin-Etsu dispensers are designed to be hand applied. Individual dispensers contain *ca* 70 mg of active ingredient and they are typically placed throughout the area to be treated at a rate of 1000/ha. In an orchard with the standard tree spacing of 6.1 m

between and within rows, 4 dispensers are attached to each tree to achieve this rate.

Although the distribution of broadcast Conrel fibres was not determined by Hathaway *et al*, it is probable that there were sources of EEAc within 1 m of the pheromone traps used to assess the trial. In the present trial traps were deliberately placed 1 m or more from the nearest source. It was considered essential that EEAc be effective at this distance when released from S-E dispensers because if an attempt were made to prevent or limit the incidence of mating in wild females, it is inevitable that some would call from positions at least 1m from the EEAc source.

Materials and methods.

Treatments. Shin-Etsu dispensers containing *ca* 70 mg of EEAc (purity not determined) were applied at a rate of 3 dispensers/4 trees (1700/ha or 119 g a.i./ha) to apple trees in a 0.29 ha plot in an orchard at Anduze in the south of France. A 0.12 ha plot adjacent to the treated plot was left untreated as the control.

Pheromone traps. Five delta pheromone traps baited with 1 mg of a 1:0.5:0.1 blend of EEOH:12OH:14OH were placed in each plot. Catches were removed on 28 occasions during the trial and baits were replaced *ca* every 30 days.

Loss rates. EEAc loss rates were determined by meniscus recession on a sample of 5 dispensers on 15 occasions during the 108-day trial (21.5.-6.9.86).

Results.

Pheromone trap catch. There was no significant difference between mean trap catch in the treated and control plots (Table 27).

Mean catch/trap	
EEAc	Control
18.6	26.0

Table 27. The effect of EEAc released as a background treatment on pheromone trap catch. There was no significant difference between means. (Student's t-test).

Loss rates. Mean loss rate was 44.8 mg/ha/h, range 8.6 - 71.6 mg/ha/h.

Discussion.

When Rothschild (1974) placed dodecenyl acetate (12Ac) at various distances from the a trap containing the pheromone of *Cydia molesta*, he found that catches were

totally suppressed when it was released from within the trap, but that the effect became less pronounced as the distance from the pheromone source increased. It was no longer significant at 60 cm or more.

In wind-tunnel trials with the lightbrown apple moth (*Epiphyas postvittana*), Rumbo *et al* (in preparation) found that males were able to land at pheromone sources in increasing numbers as the distance between an inhibitory source and the pheromone increased beyond 5 cm. Similarly, Witzgall and Priesner (1991) found that larch case-bearer (*Coleophora laricella*) males were unable to locate (*Z*)-5-dodecadien-1-ol when an inhibitor, (*Z*)-5-dodecenyl acetate, was released from the same source, but were unaffected by the inhibitor when it was placed 5 cm from the attractant.

Both EEAc and Z8-12:Ac are pheromone components of species closely related to *Cydia pomonella* - the former is found, for example, in the pea moth (*Cydia nigricana*) (Greenway 1984), the latter in *Cydia molesta* (Roelofs *et al* 1969). It is probable that in situations where these species are sympatric, mechanisms have evolved to allow *C. pomonella* males to avoid following the plumes of the wrong species. This would require that the males have antennal cells that respond to the 'inhibitors', for which there is some evidence in the case of Z8-12:Ac (Bogdanova *et al* 1980).

Thus a likely explanation for the inhibitors being effective only when in close proximity to the pheromone is that when the plumes are superimposed, as they would be when emanating from the same trap, males perceive the pheromone as coming from the wrong species and do not continue flying upwind. As the distance between the plumes becomes greater their ability to distinguish the genuine from the false trail increases, leading to a corresponding rise in trap catch.

3.3 PHEROMONE STABILITY AND RELEASE.

Introduction.

As EEOH is a conjugated diene, it is particularly susceptible to degradation by oxygen and ultra-violet (u-v) light (Shani and Klug 1980; Hoffmann *et al* 1983). To counter these effects anti-oxidants and u-v protectants such as butylated hydroxytoluene (BHT) or one of the tocophorals are usually incorporated in dispensing systems.

Observations made during the course of the mating disruption trials described in this thesis suggested that the quality of the pheromone released from the both the baits and mating disruption dispensers may have deteriorated following exposure in the field.

It was noticed that catches at traps (baited with 0.5 mg EEOH in multi-component blends) often increased dramatically (up to 50-fold) in the week following bait replacement (Section 3.1.3., Trials 1 and 2), suggesting that the baits had become less attractive during their *ca* 4 weeks exposure in the field. This observation conflicted with the results of McNally and Barnes (1980), who found that catches at rubber septa impregnated with 1 mg EEOH and aged for 1, 2, 4, 8, or 16 weeks remained constant relative to freshly-prepared baits. However although their baits were aged in the

field, they were exposed within a 'weather shelter', and it is most likely that they were largely protected from the effects of sunlight, unlike those that are placed in traps. A trial was thus established to:

1. determine whether exposure to sunlight caused the formation of products (eg geometric isomers of EEOH, Roelofs *et al* 1972) that inhibited trap catch;

2. determine whether protecting the outer surface of the baits influenced the numbers of moths they attracted relative to numbers attracted to unprotected baits. Release rates and the isomeric purity of effluvia from exposed and covered baits were also determined.

It was also observed that a thin film of pheromone began to accumulate on the external surfaces of the dispensers within a few days of them being placed in the field. Unless the anti-oxidant and u-v protectant incorporated within the dispensers had been able to pass through the dispenser walls with the pheromone, the integrity of pheromone in this secondary reservoir, ultimately the source of atmospheric pheromone, would have been adversely affected by exposure to sunlight and/or oxygen.

A noticeable feature of unexposed S-E codling moth dispensers is the straw, almost reddish colour of their contents. This disappears within 1-2 days of exposure to

sunlight and as EEOH in liquid form is almost clear, the assumption was made that the colour had been imparted by the protectants.

These observations led to speculation that the quality of pheromone being released from S-E dispensers might have deteriorated with exposure to sunlight, which in turn may have contributed to the failure of the treatments described in this thesis to disrupt mating. For this reason, trials were established to compare the proportion of EEOH in the contents and the quantity of EEOH in the effluvia of dispensers that were either exposed to direct sunlight, shaded or unexposed. In a separate trial measurements were made of the isomeric purity of effluvia from exposed and unexposed dispensers.

3.3.1 Bait formulations.

1. Formation of inhibitory products in baits exposed to sunlight.

Materials and methods.

Delta traps containing either a fresh bait ('new') loaded with 1 mg of EEOH or a fresh bait plus a bait that had previously been exposed in a trap for *ca* 30 days ('old') were put out in Stevenson's orchard. The 5 replicates of each treatment were placed on alternate

trees within two rows 12 m apart so that no replicates of the same treatment were adjacent. All 'new' baits were replaced on days 15 and 30 of the 40-day trial (1.2. - 10.3.89).

Results.

Inclusion of 'old' baits significantly reduced trap catch (Table 28).

Mean catch	
New baits	New + old baits
21.4	10.4

Table 28. Mean catch at traps baited with either 'new' or 'new' + 'old' baits. The means were significantly different at $0.01<p<0.05$. (Student's T test.)

2. Protection of the outer surface.

a. Field trials.

Materials and methods.

Treatment. Rubber tubing baits loaded with 1 mg EEOH were either wrapped in aluminium foil so that their

outer surface but not the lumen was covered ('protected'), allowing air to pass through the centre, or left unwrapped ('unprotected').

The following treatments were compared:

1. unprotected baits, changed weekly;
2. unprotected baits changed monthly;
3. protected baits changed weekly;
4. protected baits changed monthly.

The 5 replicates of each treatment were set up as a Latin square in Stevenson's orchard (the 5th treatment was of another substrate and had no relevance to the 'protected' vs. 'unprotected' aspects of this trial). Delta traps were used throughout the trial, which ran for 95 days (12.10.90 - 18.1.91).

Results.

The protected dispensers changed weekly caught significantly more moths than any other treatment (Table 29), while the unprotected baits changed monthly caught the least moths, although the mean was not significantly different from that obtained with protected baits changed monthly.

Unprotected ch'd weekly	Unprotected ch'd monthly	Protected ch'd weekly	Protected ch'd monthly
64.8 b	39.0 c	94.4 a	61.6 bc

Table 29. Mean catch/trap at unprotected or protected baits, changed weekly or monthly. Means followed by different letters are significantly different at $0.001<p<0.005$. (Duncan's MRT).

b. Determination of release rates and effluvia quality.

Explanations for the observed differences between the protected and unprotected baits include:

1. pheromone had evaporated more rapidly from the unprotected than from the protected baits, a consequence of which was that ultimately the release rates became less attractive to males than that from the protected baits;

2. shielding the outer surface of the tubing from sunlight reduced the rate at which inhibitory products formed.

Both aspects were examined in the following trials.

Trial 1. Release rates from baits.

Materials and methods.

Treatment. Six baits, each loaded with 1 mg EEOH, were chosen randomly from a stockpile of baits prepared *ca* 1 month earlier and which had been stored within an opaque container at -20°C. Three of the baits were wrapped in foil ('protected') as previously described and the other three left unwrapped.

Determination of release rates. Loss rates were determined by suspending all three baits of a given treatment within an opaque glass cylinder, through which nitrogen flowed at 60 ml/min at 26.5°C. Pheromone released from the dispensers was trapped on 60-80 mesh glass beads for 40 min.

The trap was heated to 130-140°C and the volatiles evaporated onto the front of a 12 m long, 0.32 mm o.d. BP10 column (SGE Pty. Ltd.) that had been cooled with solid CO_2. The column was then heated to 140°C and the output measured on an HP (model HP 3392A) integrator. Four collections were made for each treatment.

Results.

Wrapping the dispensers in foil reduced the release of EEOH by *ca* 40% (Table 30).

Mean release rate (ng/min)	
protected	unprotected
3.6 a	5.7 b

Table 30. Release of EEOH from rubber tubing baits either wrapped in foil ('protected') or unwrapped ('unprotected'). Means were significantly different at $p < 0.01$ (Student's T test.).

Trial 2. Isomeric purity of effluvia from baits.

Materials and methods.

Treatment. Three protected and 3 unprotected baits, as described in the previous trial, were suspended inside the same delta pheromone trap, which was placed in the field in direct sunlight.

After exposure in the field for 31 days all baits were removed, placed separately in an opaque container and stored at -20°C pending analysis.

Collection and analysis of effluvia. The effluvia were collected in the same manner as described for determining release rates. Two collections of pheromone were made from each group of three baits and they were analysed by heating the trap to 130°C under a flow of helium at 2 ml/min. Volatiles were trapped over 10 min in a 1.6 mm o.d. melting point tube by cooling it with solid CO_2.

The trap volatiles were washed out with 10 µl hexane and the solution injected onto a 25 m long, 0.32 mm o.d. BP20 column using a Grob spitless injection system. The temperature at the time of injection was 60°C and the inlet was purged after 2.5 min. After 4 min the column was heated to 170°C and the analysis conducted isothermally at that temperature.

Results

In both treatments the proportions of (*E,E*) decreased substantially compared with that of the pheromone used to prepare the baits. The effect was most pronounced in unprotected baits, and furthermore the proportion of the (*E,Z*) isomer was more than twice that of protected baits. (Table 31).

Very little pheromone remained in the unprotected baits after exposure in the field for 31 days, and although

the purpose of this trial was to measure the isomeric purity of the effluvia, the methods used in doing so allowed estimates to be made of pheromone release rates. They were 49.9 pg/min and 153.3 pg/min in the unprotected and protected baits respectively. (All isomers).

Treatment	Isomer proportion (%)			
	ZE	EE	EZ	ZZ
protected	12.8	75.0	12.1	-
unprotected	13.3	56.2	30.4	-
source pheromone	1.2	96.4	2.4	-

Table 31. Proportions of isomers detected in the effluvia of pheromone baits exposed in the field for 31 days. The proportions of isomers in the source pheromone are presented for comparison.

Discussion.

Maitlen *et al* (1976) reported that the half-life of rubber stoppers impregnated with 1 mg of EEOH was 26.5 days, and that after 11 weeks 0.125 mg remained. In a trial comparing the attractiveness of stoppers charged with 0.0001 - 10.0 mg EEOH, McNally and Barnes (1980) found that 0.1 and 1.0 mg loadings consistently caught the most moths and so it seems unlikely that sub-optimal release rates from the unprotected dispensers (relative

to the protected) contributed to the differences in catch, which were already apparent after one week.

A more plausible explanation for the differences is that the (*EZ*) isomer inhibited trap catch, the formation of which was retarded by covering the outer surfaces of the baits with foil. This isomer, as well as the (*ZE*), is known to inhibit catch (Roelofs *et al* 1972), but as the proportions of the latter were similar in both the protected and unprotected baits, it cannot have been responsible for the observed differences. The effects of isomerization were also noticed by Cork *et al* (1988), who reported that synthetic lures containing the conjugated diene aldehyde (*E,E*)-10, 12-hexadecadienal had to be protected from sunlight to prevent loss of attractiveness caused by isomerization. This was achieved by covering the pheromone dispensers with aluminium sleeves.

3.3.2. Disruptant formulations.

In Trials 1 and 2 the proportion of EEOH relative to other isomers in the contents and the rate of EEOH release from S-E dispensers that were either shaded or exposed to direct sunlight was measured. In the third trial the proportion of EEOH relative to the other isomers in the effluvia from a dispenser exposed to direct sunlight was determined and compared with that from an unexposed dispenser.

Trials 1 and 2. EEOH in contents and release rates from exposed, shaded and unexposed S-E dispensers.

Materials and methods.

Treatment. Ten S-E dispensers loaded with *ca* 70 mg EEOH were suspended in direct sunlight ('exposed'). Another 10 were folded in half, hung centrally within 30 cm lengths of 15 mm (i.d.) metal tubing ('shaded') and suspended beside the 'exposed' dispensers. The tubes were white on the outside to reflect light and black on the inside to absorb it. Corks were inserted in the tops of the vertically-hung tubes to prevent light entering from above, the bottom being left open to allow limited air exchange. A control group of 'unexposed' dispensers was wrapped in foil to exclude light and kept in the laboratory at room temperature.

At fortnightly intervals the effluvia and contents from one unexposed, two exposed and two shaded dispensers were analysed.

Temperature measurements. Temperatures within a Stevenson screen and inside the tubes were measured at hourly intervals with thermocouples linked to a data logger. Mean temperatures for the period of the trial (6.6 - 13.8.89) were calculated from the average daily maximum and minimum temperatures.

Effluvia collection and analysis. A glass collection tube (55 mm long, 4 mm i.d., but narrowed to 2 mm i.d. over a 10 mm length of one end) was packed with *ca* 200 mg of 60-80 mesh glass beads that were retained within the tube by stopping its ends with glass wool.

One end of the tube was positioned as close as possible to a portion of the dispenser containing a continuous column of pheromone without actually touching it, while the other end was connected to a vacuum pump that drew air through the trap over 10 min collection periods at 20 l/min.

Volatiles trapped on the glass beads were desorbed in a heated oven at 130°C under a stream of helium in a specially constructed inlet system (Whittle, unpublished data), and subsequently trapped over 10 min in the cooled (dry ice) front portion of an SGE Pty Ltd BP10 column. The cooled portion of the column was moved into the injection port that was held at 240°C and the quantity of EEOH collected was then determined by isothermal chromatography at 140°C.

Contents analysis. All dispensers were placed in a room at 32°C for 10 minutes immediately before sampling to ensure that the contents were liquid. A *ca* 10 mg aliquot was then removed with a syringe, weighed and combined with a similar quantity of tetradecyl acetate (14 Ac).

Two to five samples of this mixture were then analysed on a BP20 column at 170°C to determine the ratio of EEOH to 14 Ac.

Results.

Quantity of EEOH in the effluvia. It proved very difficult to standardise the collection of effluvia between sampling periods, largely because minor differences in the position of the collector had considerable influence on the quantity of EEOH collected. This led to measurements of large fluctuations within treatments over time, despite which a consistent trend emerged to indicate that the greatest quantity of EEOH was recovered from the unexposed, followed by the shaded and the exposed dispensers (Fig. 6).

Contents analysis. Mean % EEOH varied considerably between sampling periods for a given treatment, with no particular trend being evident (Fig. 7). There were no significant differences between treatments in terms of the means of all samples taken during the trial (Table 32).

Temperature measurements. Mean temperatures in the screen and tube were, respectively, 7.5°C ± 2.2 s.d. and 7.1 °C ± 2.2 s.d.

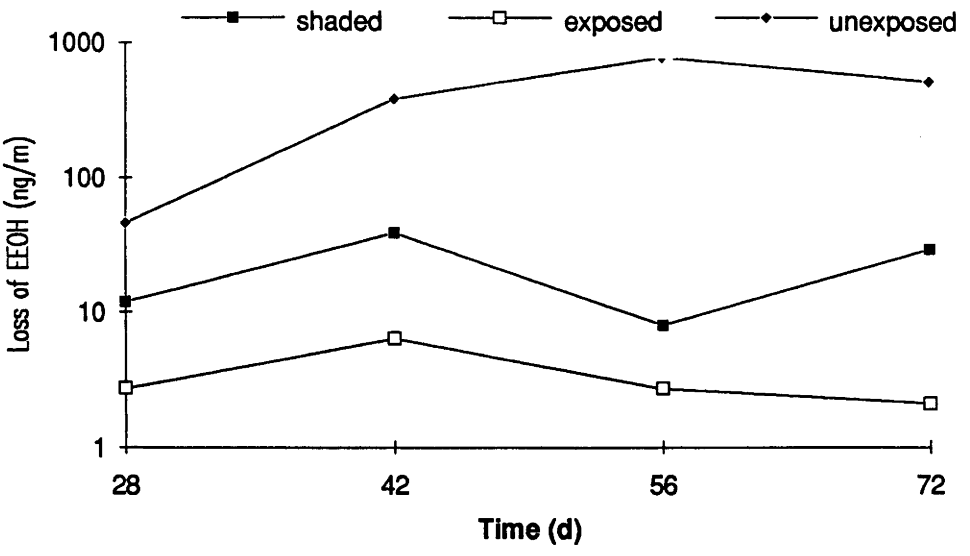

Fig. 6. Quantity of EEOH recovered in the effluvia from shaded, exposed and unexposed Shin-Etsu dispensers.

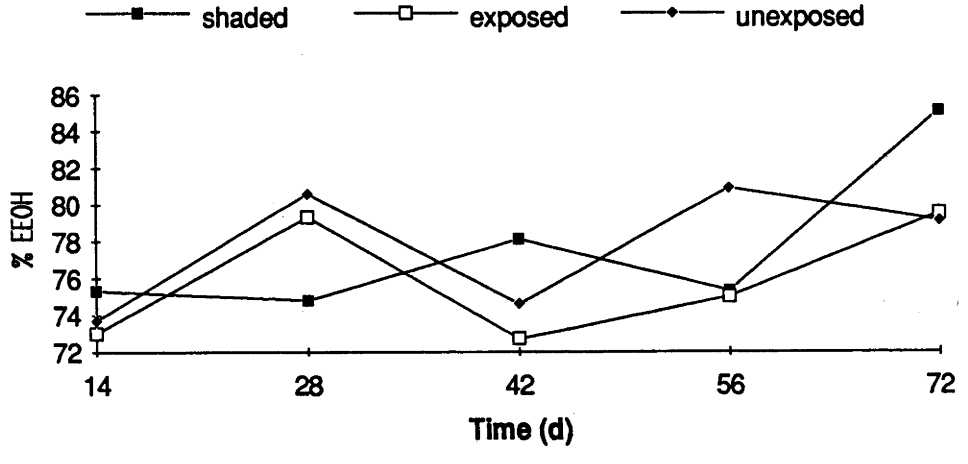

Fig. 7. % EEOH in the contents of shaded, exposed and unexposed Shin-Etsu dispensers.

Unexposed			Shaded			Exposed		
mean	(n)	s.d.	mean	(n)	s.d.	mean	(n)	s.d.
77.0	(25)	3.8	78.5	(42)	5.9	75.6	(36)	4.0

Table 32. % EEOH recovered from the contents of S-E dispensers that were either unexposed, shaded or exposed to light. There were no significant differences between means. (Duncan's MRT).

Trial 3. Isomeric purity of EEOH in exposed and unexposed S-E dispensers.

Material and methods.

Treatment. An S-E dispenser loaded with *ca* 70 mg EEOH was suspended in direct sunlight for 84 days (13.9 - 5.12.91), then taken down and immediately placed in the apparatus for collecting effluvia. Effluvia collections were subsequently taken from an unexposed dispenser that had been stored at 4°C in total darkness. Both dispensers were left to equilibrate in the apparatus for 1 h before collections began. Two collections were taken from the exposed dispenser and three from the unexposed. No records of temperatures were kept during the period that the exposed dispenser was in the field.

Collection and analyses of effluvia. The method used was as described for the collection and analysis of effluvia from baits (Section 3.3.1.) except that collections were made over 2 min because of the greater quantity of pheromone being released.

Results.

There were no significant differences between the exposed and unexposed dispensers in terms of the proportion of (*EE*) in the effluvia. Exposure to sunlight caused the conversion of small quantities of the (*ZE*) isomer to (*ZZ*), whilst the proportions of (*EZ*) were similar in both treatments (Table 28).

Treatment	Isomer			
	ZE	EE	EZ	ZZ
Exposed	6.5 a	84.2 a	7.3 a	2.9
Unexposed	8.0 b	84.8 a	7.2 a	0.0

Table 33. Mean % of isomers in the effluvia of a Shin-Etsu dispenser exposed to direct sunlight for 84 days compared with an unexposed dispenser. Means within a column not followed by the same letter are significantly different at $p < 0.01$. (Student's t test)

Discussion.

Interpretation of the release rate results must be tempered with caution, for although they suggest that EEOH was released from dispensers exposed to sunlight at much lower rates than it was from the unexposed and shaded dispensers, it is possible that some of the observed differences were caused by there being a greater accumulation of EEOH on dispensers around which air flow had been eliminated (unexposed) or restricted (shaded) prior to collections being made.

Incorporation of conjugated dienes in polyethylene is suspected to accelerate their decomposition when exposed to light and air (Shani and Klug 1980; Hoffmann *et al* 1983). However the results of the present trials indicate that the integrity of EEOH was maintained both within the S-E dispenser (made of polyethylene and polyvinyl acetate) and on its outer surface.

The discrepancy between proportions of EEOH measured in the contents (Trial 1: 77.0%) and effluvia (Trial 3: 84.8%) of unexposed dispensers may be explained by the fact that the analyses were made at different times and the dispensers subjected to different conditions. Both proportions are lower than one might expect of pheromone that had not received any exposure to light, but as no measurements were made of isomeric purity when the dispensers were first received (September 1986), it is

not possible to say whether impure materials had been used during formulation or whether there had been some loss of purity during storage.

3.4. ANALYSIS OF FEMALE EFFLUVIA.

Introduction.

Release of a 3-component blend failed to limit the incidence of mating (Section 3.1.1.3) and evidence from behavioural trials indicated that a combination of EEOH, 12OH and 14OH was no more effective than EEOH alone in either interfering with the ability of males to locate synthetic baits when released as a background, or in eliciting male sexual activity in a laboratory bioassay. (Section 3.1.4).

The latter findings contradict those of Bartell *et al* (1988) and raise doubts about the validity of their claims that 12OH and 14OH were the behaviourally-active components (other than EEOH) whose presence had previously been indicated in the bioassays of Bartell and Bellas (1981).

Further doubts arise when the data identifying the chemical nature of the fractions are re-examined. Of the two female extract fractions other than EEOH separated on a polar stainless steel column containing 10% PEGA on Diatomite C, one appeared before (1st fraction) and one after (3rd fraction) the position occupied by EEOH (Bartell and Bellas 1981). A subsequent paper indicated that the 1st and 3rd fractions were, respectively, 12OH and 14OH

(Bartell *et al* 1988). However in that paper both 12OH and 14OH eluted before EEOH in an analysis in which a polar column (SP 1000) was again used, and thus the attribution of 14OH to fraction 3 appears to be erroneous.

The failed mating disruption trials, an inability to demonstrate enhanced behavioural activity in blends containing 12OH and 14OH and a re-interpretation of the original analyses all indicated a need to re-examine the composition of the pheromone. However an analysis of pheromone effluvia rather than gland contents was considered to be more appropriate in the context of attempts to develop a mating disruptant whose composition resembled that released by the female.

Arn *et al* (1985) analysed effluvia content, but their methods can be criticized on a number of grounds. The duration of the scotophase was not stated, but on the basis of the paper describing the rearing method used (that of Mani *et al* 1978b), was probably 6 hours. During this period and the 30 minutes before scotophase began, there would have been considerable opportunity for pheromone accumulating on the surfaces (the internal walls of a glass bottle and the bodies of the moths (Baker *et al* 1980; Ramaswamy and Carde 1984)) to be degraded by oxidation and by the action of enzymes from body scales (Morse and

Meighen 1984, Silk *et al* 1985). The latter would also have been deposited intact on the glass collection surfaces. Steps were taken in the present study to avoid these difficulties.

Materials and methods.

Insects. All females were derived from the 2-4th generations of a laboratory culture established with field-collected adults. They were sexed as pupae and allowed to emerge in a light box running on a 14:10 L:D cycle, and in which they remained until required for collections of effluvia. Emergences were noted daily and only females aged between 2 and 5 days were used. None were used more than once.

Collection apparatus and procedures. A manifold apparatus for making simultaneous collections of airborne pheromone from up to 20 individual free-moving moths was described by Shani and Lacey (1984). A gentle air stream (Medical Grade at 30 ml/min) was passed over each unrestrained moth in its own glass cage and the effluvium allowed to flow through the capillary section of a flame-cleaned Pasteur pipette. Aerially released pheromone molecules adhered to the walls of the capillary and were recovered at the conclusion of the collection period with microlitre volumes of HPLC grade solvents such as pentane or hexane.

The original procedure (Shani and Lacey 1984) was modified in two ways to enable collection of airborne pheromone from codling moth. Firstly, air was drawn through each capillary trap at 25 ml/min to alleviate turbulence within the cage, which otherwise would have led to competitive retention of pheromone by the walls of the cage. Secondly, the capillary trap in each case was moved as close as possible to the everted gland of the calling (ie pheromone-releasing) female so that, ideally, the flared entrance of the trap enveloped the gland. In later experiments, the capillary trap was curved slightly near the flared orifice to facilitate manipulation of the moth-to-trap distance within the narrow confines of the cage.

The temperature ranged between 23-25°C at the time females were placed in the apparatus but was reduced to 18-20°C at the beginning of scotophase to encourage the onset of calling. Observations began with scotophase and duration of calling was noted for each female. When all calling had ceased, generally within 1.5 hours of the beginning of scotophase, the capillaries containing trapped pheromone solutions were sealed and stored at -5°C until required for measurement by gas chromatography/mass spectrometry (GC/MS).

Measurement of pheromone. The technique used for pheromone determinations was as described by Lacey and Sanders (in press) for the analysis of effluvia from *Cydia molesta*. The following description has been taken from that account.

"GC/MS was carried out on a VG 70-70 mass spectrometer (VG Analytical, Manchester) interfaced directly to an HP 5729A capillary gas chromatograph (Hewlett Packard, CA) and a VG 11-250 data system. Solutions for analysis were introduced by way of an HP 19290A on-column injector. The cross-linked chromatography columns used (DBWax, J&W, 30 m length, 0,32 mm i.d., 0.5 µm phase thickness; BP20, SGE, 25 m length, 0.33 µm i.d., 1.0 mm phase thickness) were preceded by retention gaps of deactivated fused silica (SGE, 4 m length, 0.33 mm i.d.).

The on-column GC methodology used here to analyse the effluvia enabled transfer of mixtures through the gas chromatograph with minimal molecular discrimination (Grob, 1987). The use of a retention gap as a fore-column preserved the column's integrity and permitted the injection of large volumes without seriously jeopardising the chromatographic resolution. Large injection volumes allow the full dynamic range of sensitivity to be exploited because the

concentration of solutions to very small volumes often leads to major loss of the solutes on surfaces or by evaporation. While very long retention gaps are normally prescribed for accommodating large injections (Grob, 1987), it was found in the present work that equivalent results could be obtained by injecting small aliquots (2-3 ml) in sequence onto a short retention gap such that the solvent evaporated between injections. The high conductance of the source diffusion pump was adequate to dispose of the vapour without electrical discharge.

The GC columns and retention gaps were reserved for effluvium analysis. Helium (Ultra-high purity, CIG Australia) was used as a carrier gas and a typical temperature program for elution of the pheromone constituents was 30°C isothermal followed by a gradient of 30°C/min to 165°C isothermal (DB Wax). Injections of samples could be accomplished at 20-25 min intervals under such conditions.

The ionisation mode adopted for determination of the pheromone components in the present study was negative-ion chemical ionisation (NICI) using hydroxyl ions as the reagent. The OH^- ions were formed in the source by the addition of nitrous oxide (Instrument Grade, CIG Australia) to

methane (Ultra-high purity, CIG Australia) at a combined pressure of 40 Pa. The ionisation energy was 100 eV, the source temperature 190°C and the filament emission 1 mA. Selected ion monitoring of $(M-H)^-$ ions was accomplished at constant magnetic field by alternation of the accelerating and electric sector voltages under data system control, with dwell times of 75 msec per channel."

Results.

Mean pheromone yield/calling female was much lower than had been anticipated (0.13 ng/h/female, n = 8) on the basis of those from other species using a similar method. *Ephestia cautella* for example yielded 0.65 ng/h (Barrer *et al*, 1987) and *Cydia molesta* 9 ng/h (Lacey & Sanders, in press). Because individual yields were so low, components present in the effluvia in quantities of 10×10^{-12}g or less would not have been detectable and collections from individuals were amalgamated, using only those from females seen to call for at least 1 hour (n = 10).

The mean yield was 0.54 ng. Ratios of the components are shown in Table 34. Major differences between the two analyses are evident in terms of the relative quantities of E9-12:OH, (*E,Z*)-8,10-dodecadien-1-ol (EZ12:OH) and 12:OH

Discussion

There are no obvious reasons for the observed differences in the content of E9-12:OH, which is almost certainly a pre-cursor of EEOH (Lofstedt and Bengtsson 1988) and which has no demonstrated behavioural role. EZ12:OH was detected only by Arn *et al*, albeit in small quantities, and the compound may have been formed as a consequence of light falling on the trapped effluvia, causing isomerisation of some of the EE12:OH.

Component	Ratio	Arn *et al*
12:OH	23	643
E9-12:OH	<10	143
EE-8,10:12Ac	<2	-
EE-8,10:12OH	1000	1000
EZ-8,10:12OH	-	48
14:OH	178	95
16:OH	222	190
18:OH	115	214

Table 34. Component ratios relative to EEOH detected in effluvia collected individually from 10 virgin females and amalgamated for analysis. The results of Arn *et al* (1985) are presented for comparison.

It is possible that differences in solvent purity and quantity used, and contamination of the collection by insect scales contributed to differences in quantities of 12:OH detected, and indeed to the generally high levels of saturated alcohols in both analyses. It became apparent from analyses of individual effluvia collections that the quantities of saturated alcohols were mainly independent of the amount of EEOH. They were also detected in females that had apparently not called during the collection period, suggesting that there was a source or sources of the compounds other than the pheromone.

Tests of males placed in the same apparatus and of extracts from wing and body scales of virgin females revealed that both contained 12-18 carbon saturated alcohols. Lipids in insect cuticle are known to contain saturated alcohols (Lockey, 1988) and were a likely source in these analyses.

A common ingredient in all collections was the solvent hexane, analyses of which indicated that it contained an homologous series of the saturated alcohols at concentrations of less than 1 part/billion (Lacey and Sanders, in press). Once the solvent was concentrated, as it was in the course of preparing the collections for analysis, the saturated alcohols would have been enriched, particularly those

with higher boiling points such as the 16 and 18 carbon compounds.

Thus analyses in which hexane has been used and/or in which the collection may have been contaminated by insect scales, and that indicate the presence of saturated alcohols (eg Arn *et al* 1985), must be treated with caution. It could of course be argued that even if the saturated alcohols were derived from the body scales, they should still be considered constituents of the effluvia. However it seems unlikely that under these circumstances they would have any behavioural effects, and certainly none have been demonstrated in the present trials.

3.5. THE EFFECT OF DELAYED MATING ON OVIPOSITION PATTERN, FECUNDITY AND FERTILITY.

Introduction

Whilst the primary aim of mating disruption treatments is to reduce the incidence of mating and so limit the size of succeeding generations, a similar although less pronounced effect may be achieved by delaying mating, providing it leads to a reduction in the fecundity and/or egg fertility of females and, as a consequence, fewer larvae reaching adulthood.

A delay in mating has been shown to adversely influence the reproductive potential of several Lepidoptera species. Barrer (1976) found that it reduced fecundity and egg fertility in *Ephestia cautella*. Similar effects were demonstrated in *Homona magnanima* (Kiritani and Kanoh 1984), *Pectinophora gossypiella* (Lingren *et al* 1988) and *Chilo partellus* (Unithan & Payne 1991). Ellis and Steele (1982) demonstrated reduced fecundity in *Spodoptera littoralis* by delaying mating, as did Proshold *et al* (1982) in *Heliothis virescens*.

In the present study mating was artificially delayed in both males and females to determine if there was any effect on the fecundity, egg hatch and oviposition pattern of females. From the data so gathered the net reproduction rate (R_o) was calculated. This value takes

into account age-specific mortality, age-specific oviposition and the proportion of eggs that hatch. It provides a measure of the rate of increase within a single generation.

No account was taken of potential differences in the probability of 0, 2 or 4-day old females and 2 or 6-day old males mating. In this respect the influence of age is likely to be minimal. Howell *et al* (1978) found that 24 h old virgin females mated as readily as those that were 72-96 h old, although 1-day old males were more likely to mate than 2 to 7-day old males.

Materials and methods

Delayed mating of females. Male and female pupae from the laboratory culture were kept in separate containers. Emerged adults were collected daily and females placed individually in egging cups with a supply of 5% sucrose solution.

A 2-3 d old male was placed in the container with a female on the day of her emergence (day 0) or 2 or 4 d after emergence. The male was removed 24 h later.

Fecundity was measured by noting the number of eggs laid by each female daily from day 0 until she died, after which she was dissected and classified as being mated or

unmated on the basis of the presence or absence of a spermatophore in the bursa copulatrix.

The effect of delayed mating on fertility was determined by separately collecting all eggs laid 1-4 d (inclusive) after mating and those laid from day 5 onwards, storing them at 25°C for 5 d and then examining them to determine the proportion that had hatched. The eggs from *ca* 20 females in each of the three age groups were collected for this purpose.

R_o was calculated by determining the age-specific fecundity rates for females in each of the 3 treatments (Birch 1948). No account has been taken of the cumulative effect that a delay in mating might have over successive generations.

Delayed mating of males. The materials and methods used were as described above, except that 2 males (either 2 or 6 d old) were paired with each 2 d old female. All eggs were retained to determine the proportion that hatched.

All experiments (with both males and females) were conducted at 24 ± 2°C and 60 % r.h. in a 14:10 L:D cycle.

Analyses. All data were analysed with a 1-way ANOVA, but with an arc-sine transformation of the % egg hatch

values. The significance of differences between means was obtained from least significant differences.

Results.

Females - *fecundity, oviposition pattern and longevity.* Delayed mating had a significant effect on both mean fecundity and on the proportion of eggs that hatched, but not on female longevity (Table 35). Oviposition pattern was similar for all treatments and is reflected in Fig 8, which depicts the production of female larvae/adult female.

Net reproduction rate. Net reproduction rate was not substantially affected when mating was delayed by 2 d, but was reduced by 45% in females mated 4 d after emergence. (Table 35). The greatest contribution to net reproduction rate occurred within 7 days of mating (Fig. 8), during which period >92% of first instar larvae were produced, regardless of treatment.

Males.

Delayed mating of males had no effect on the fecundity or proportion of eggs that hatched. The results are presented in Table 36.

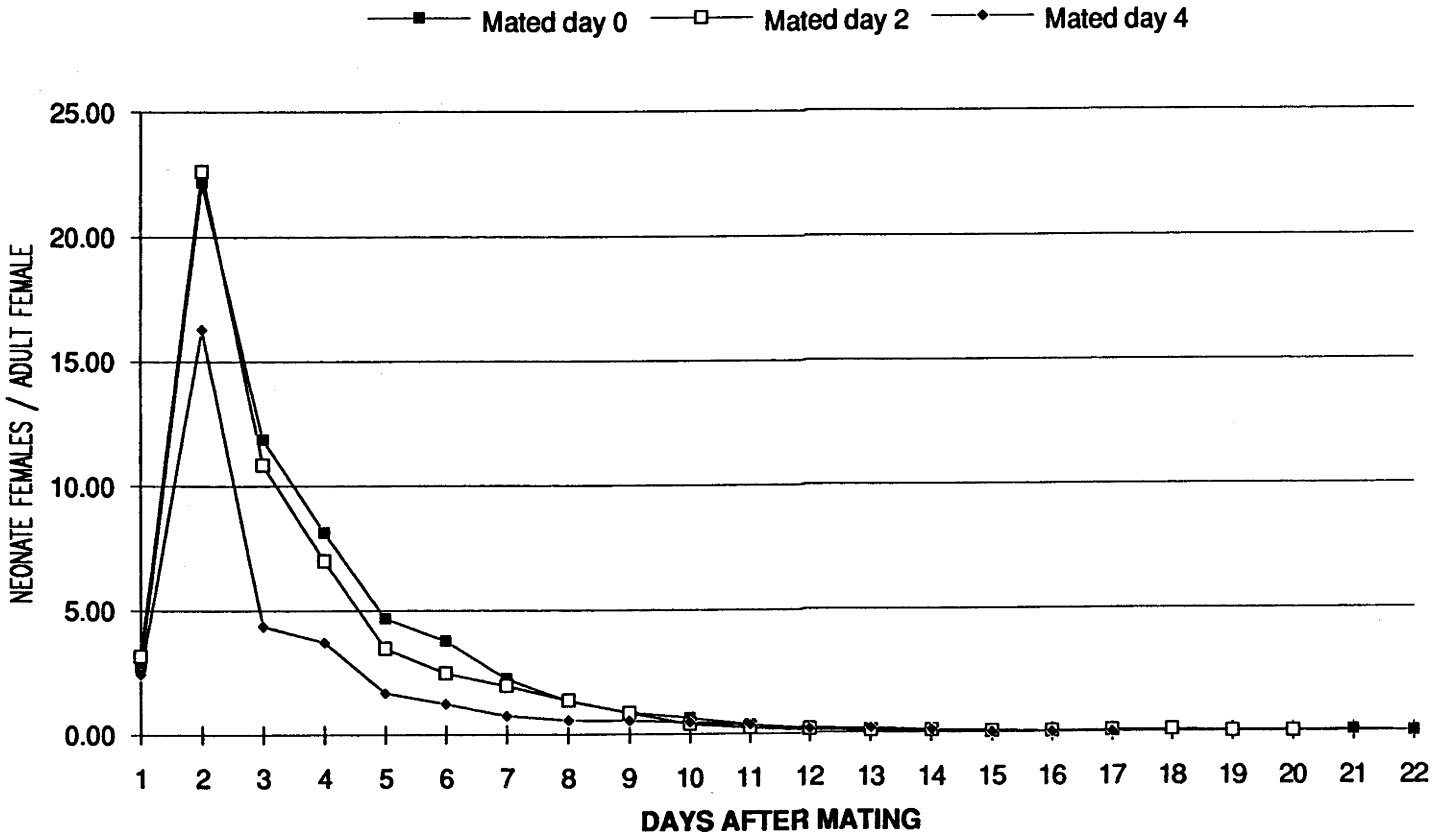

Fig. 8. The effects of delayed mating on the production of female larvae/adult female.

Measure	Age when mated (d)		
	0	2	4
Mean fecundity *	158.3 a	146.7 a	114.8 b
Mean longevity	10.1 a	11.5 a	12.4 a
% eggs hatched after mating:			
1-4 days **	80.9 a	82.7 a	61.5 b
5+ days	61.4 a	53.8 a	49.2 a
Net reproduction rate	59.4	54.7	32.8

Table 35. Effect of delayed mating of females on fecundity, longevity, % egg hatch and net reproduction rate. Means not followed by the same letter for a given measure are significantly different at * p = 0.05; ** p = 0.001

Male age	Mean fecundity of 2-d old females	% hatch during days 1-4	5+
1	161.0	80.7	71.3
6	139.9	83.5	78.6

Table 36. Effect of delayed mating of males with 2-d old females on fecundity and % egg hatch. Mean fecundity and % hatch were not significantly different between treatments. (Student's T test).

Discussion.

The difference in R_o values between females mated on the day of emergence and after 4 days will be exacerbated by reductions in longevity. If, for example, mean female longevity had been 8 days, then the R_o value for females mated 4 days after emergence would have been reduced by 53% compared with that of females mated on the day of emergence.

Data on the longevity of female codling moth in the field is limited, although Geier (1963) found that those confined to sleeve cages lived for a mean of 12 days, a similar period to that established for females under laboratory conditions in the present study. However the former were not subjected to predation or the need to fly and expend energy (as free-flying females in the field would) and their longevity has probably been over-estimated. For this reason it is likely that the imposition of a treatment that delays mating in the field will have a greater effect on R_o than was demonstrated in the laboratory.

The effect of delayed mating will be most pronounced in situations where mortality of the immature stages is unaffected by limited availability of resources such as food and shelter, as is likely to be the case in low

density populations. Where these resources are limited, the resultant increase in mortality will to some extent negate the beneficial effects of delayed mating.

4. CONCLUSIONS AND DISCUSSION

The aims of the research undertaken for this thesis were to:

1. assess the potential of multi-component pheromone blends and two pheromone-related compounds known to inhibit trap catch to disrupt mating;

2. identify the components in female effluvia;

3. investigate the stability of pheromone contained in and released from the dispensing system used in most of the mating disruption trials;

4. determine the effect of delayed mating on the net reproduction rate of codling moth.

The results of the research can be summarised as follows:

Evaluation of single and multi-component blends.

1. Multi-component blends were no more attractive to males than EEOH alone. (3.1.1)

2. Suppression of trap catch was influenced by the distribution of pheromone release points within multi-

tree plots. Three and 4-component blends were equally effective in suppressing catch. (3.1.2)

3. Suppression of trap catch was not influenced by distribution of pheromone release points within individual trees. (3.1.2)

4. Neither of the multi-component blends tested were effective as mating disruptants. (3.1.3)

5. There was no significant difference in the numbers of males able to orientate towards and land near a synthetic pheromone source in air permeated either with EEOH or EEOH +12OH + 14OH. (3.1.4.1).

6. Activity levels at dusk of males confined to air permeated with EEOH were significantly lower than those confined to pheromone-free air. (3.1.4.1)

7. The addition of 12OH and/or 14OH to EEOH did not enhance male sexual activity in the laboratory bioassay and evidence indicating a behavioural role for 12OH (other than at sub-optimal EEOH levels) and 14OH is rejected. (3.1.4.2)

8. Claims that whole female extract contains behaviourally-active components other than naturally occurring EEOH could not be confirmed in a repeat of the original bio-assay. (3.1.4.2)

Evaluation of pheromone-related compounds.

1. The ability of Z8-12 Ac to suppress trap catch was inversely related to its distance from EEOH. It had no significant effect on trap catch when placed 600 mm or more from EEOH or when released as a background treatment. (3.2.1.1)

2. EEAc released as a background treatment had no significant effect on trap catch. (3.2.1.2)

Analysis of female effluvia.

1. Whilst claims that saturated alcohols are constituents of the effluvium cannot be dismissed, it is probable that some, if not all were derived from the solvents used in the analyses and/or from moth scales that contaminated the effluvia samples. (3.4)

Pheromone stability and release.

1. EEOH integrity was maintained within and on the outer walls of the dispensing system used in most of the described trials. Release rates of EEOH may have been affected by exposure of the dispensers to sunlight. (3.3.1)

2. Protecting pheromone baits from sunlight increased

their longevity. Baits exposed in the field for *ca* 30 days inhibited trap catch when placed beside fresh baits. (3.3.2)

Effects of delayed mating.

1. Net reproduction rate was adversely affected by delays in the mating of females, but not of males. The effect was achieved by a combination of reduced oviposition and egg hatch. (3.5)

2. The longevity of females was not affected by delays in mating of up to 4 days. (3.5)

Discussion.

Published evidence for there being two or more behaviourally-active components in the pheromone of codling moth must now be regarded with some suspicion. The finding that saturated alcohols occur naturally in the organic solvents used for analyses and in body scales casts doubt on claims that they are components of the effluvia. If they are present, evidence gathered from the trials described in this thesis indicates that they have no behavioural function. Furthermore, in a repeat of bio-assays undertaken by Bartell and Bellas (1981), the claim that whole pheromone gland extracts induced greater levels of sexual activity in males than

fractions of the extracts containing only natural EEOH could not be substantiated.

Further support for the contention that EEOH is the only behaviourally active component of codling moth pheromone is provided by the findings of Rumbo (personal communication, 1989), who surveyed the male antenna for cells responsive to the saturated alcohols 12OH, 14OH and 16OH, the dienes E7- through to E10-12OH and the diene (*E*) and (*Z*) alcohols with double bonds at positions 7 and 9 and 8 and 10. He obtained no significant responses to the saturated alcohols or dienes at concentrations commensurate with those present in the pheromone gland. Cells that were responsive to EEOH also responded to E8-12:OH and E10-12:OH, but neither have been identified in the gland or effluvia and did not induce activity in a 'wheel' bioassay. Rumbo concluded that the population of receptors on the male antenna was essentially homogeneous, which when challenged with components of the pheromone gland responded only to EEOH.

Thus in terms of composition, EEOH alone should be sufficient to disrupt mating. Codling moth is a relatively sedentary species with a restricted host range, both of which characteristics suggest it should be amenable to control by mating disruption and indeed Charmillot (1990) and Mani *et al* (1984) have demonstrated that under Swiss conditions release of EEOH

has provided satisfactory control. Nevertheless both authors have also reported occasional failures, the most recent of which was attributed to the inability of the dispensing system to release sufficient pheromone. (Mani, personal communication, 1991).

The Shin-Etsu dispenser appears to be capable of protecting its contents and pheromone accumulating on the outer surface from the degradative effects of sunlight and oxygen. However even if it were not, and a mixture of isomers were released, the level of disruption may not be affected. Despite the finding that geometric isomers of EEOH inhibited trap catch (Roelofs *et al* 1972), Charmillot (1990) reported that mating disruption trials with a crude mixture containing about 70% of the (*EE*) isomer gave results comparable to those achieved with pure EEOH. The same impure product was ineffective as a bait in traps.

An impediment to our understanding of mating disruption mechanisms concerns a lack of knowledge about aerial concentrations of pheromone and its distribution. Borden (1931) noticed that codling moth flight was concentrated near the tops of apple trees. Trials to determine optimum pheromone trap placement have indicated that catches increased with trap elevation within the canopy (Riedl et al 1979, McNally and Barnes 1981). These findings suggest that considerable mating activity takes place in the upper canopy and although other researchers

have found no significant effect of trap height on catch (Thwaite and Madsen 1983, Howell *et al* 1990), it would seem prudent to ensure that for mating disruption purposes pheromone is distributed as evenly as possible throughout the canopy.

Although this can be achieved in the short term by placing dispensers in appropriate positions within the canopy, under some meteorological conditions pheromone may be quickly removed from the region by wind and/or turbulence, creating a relatively pheromone-free zone in which the behaviour of males will be unaffected by the imposition of mating disruption treatments. Similar effects are likely to occur on the windward boundaries of pheromone-treated orchards.

There are currently no means of measuring aerial concentrations and distribution other than those that rely on collecting samples over several hours (Caro *et al* 1981, Flint *et al* 1990). As male behaviour is influenced by instantaneous fluctuations in concentration (Wright 1958, Aylor *et al* 1976, Elkington *et al* 1984), time-averaged measurements are of little value in determining the likely effects of dispenser placement and pheromone release rates on male behaviour. The portable electroantennograph developed for measuring pheromone plume structures in the field (Baker and Haynes 1989) has potential as a means of obtaining information about instantaneous concentrations and

distributions and warrants further development for these purposes.

Mating disruption is thought to be population density dependent (Beroza and Knipling 1972, Knipling 1979), for which there is now field evidence (Carpenter *et al* 1982, Webb *et al* 1988, 1990). As previously indicated, Mani (unpublished report) concluded that in relation to mating disruption of codling moth 'the larval population should be as low as possible, certainly below 1000 larvae/ha in the year before pheromone application starts' for the method to succeed.

The number of mature larvae/ha that would result in 2% of an apple crop suffering from deep entries is calculated to be *ca* 1300 on the basis of values derived by Geier (1963) for the mean number of apples/tree (544) and deep entries/adult female (15), and for mortality rates between mature larvae and adults (77.3%, Wearing 1979). On a standard planting of 270 trees/ha this is equivalent to 4.8 larvae/tree.

If Mani's value of <1000 larvae/ha is correct then it would appear that mating disruption of codling moth is only likely to succeed in situations where populations are already low and damage to the crop is <2% It will probably be necessary in many situations to supplement disruption treatments with insecticides to reduce the population to a level at which it can be effective, but

there is no evidence to suggest that the addition of pheromone gland components other than EEOH will make a more efficacious disruptant.

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6. APPENDICES

6.1. Orchard descriptions

Banks'

Location: Canberra, ACT. Situated in a small apple-growing district with potential sources of infestation within 500 m.

Area: 1.8 ha.

Varieties: Apples: Cox's Pippin, Democrat, Golden Delicious, Granny Smith, Jonathon, Macintosh Red, Queen of the South, Red Delicious, Yates. Ages vary from 2 - 20 years. Tree height 2.5 - 4.0 m.

Tree spacing: 4.0 m within rows, 4.8 m between rows.

Previous history: A commercial orchard that has received regular applications of azinphos-methyl for codling moth control for at least the past 6 years.

Stevenson's.

Location: Canberra, ACT. At least 1 km from the nearest external source of codling moth.

Area: 1.7 ha.

Varieties: Apples: Rome Beauty, Granny Smith and Red Delicious, all *ca* 50 years old. Tree height 2.5-3.0 m.

Tree spacing: 6.1m x 6.1m

Previous history: This orchard has been used for experimental purposes for at least 25 years. It has not

received any insecticide for the past 20 years and consequently supports a high codling moth population.

French orchard

Location: Anduze, *ca* 50 km west of Montpellier, southern France. At least 1 km from the nearest external source of codling moth.
Area: 0.4 ha.
Varieties: Apples: Not determined. Tree height 2.0-2.5 m.
Tree spacing: Mostly 1.75 m within rows, 4.0 m between rows.
Previous history: A commercial orchard that received irregular applications of azinphos ethyl. Codling moth population light-moderate.

Georgeovitch's

Location: ca. 20 km from Canberra. At least 1 km from nearest source of infestation.
Area: 0.3 ha.
Varieties: Apples: Semi-dwarf Golden Delicious, Granny Smith and Red Delicious. Tree height 2.0-2.5 m.
Tree spacing: 2.5 m within rows, 5.0 m between rows.
Previous history: A 'hobby farm' that has received irregular applications of azinphos methyl. Codling moth population is moderate.

Spurway's

Location: Orange, NSW. Situated in a large pome-fruit growing region. Adjacent to another orchard.
Area: 4 ha. (Apples: 2 ha., Pears: 2 ha.).
Varieties: Apples: Granny Smith; Pears: Burre Bos. Tree height 2.5-3.5 m.
Tree spacing: mostly 6.1 x 6.1.
Previous history: A commercial orchard that has received regular applications of azinphos methyl. Codling moth population is moderate.

Wood's

Location: near Barry, NSW. At least 10 km from the nearest source of infestation.
Area: 5.8 ha. (apples, 3.7 ha; pears 2.1 ha.).
Varieties: Apples: Granny Smith, Red Delicious; Pears: Burre Bos, Packham. Tree height 2.5-4.0 m.
Previous history: A commercial orchard that has received regular applications of azinphos methyl. Light to moderate codling moth population.

p p p p p
p Δ . Δ p
p . Δ . p
p Δ . Δ p
p p p p p

3 disp./tree on 16 trees

p p p . p
. Δ . Δ p
p . Δ . p
p Δ . Δ .
p . p p p

4 disp./tree on 12 trees.

p . . . p
. Δ . Δ .
p . Δ . p
. Δ . Δ .
p . . . p

8 disp./tree on 6 trees.

Appendix 6.2. Location of dispensers and pheromone traps in Spurway's orchard, Trial 2, Section 3.1.2. p = tree with pheromone dispensers: Δ = pheromone trap.

6.3 Dispenser descriptions and pheromone loss rate calculations.

Dispensers.

Shin-Etsu. A 'double-barrelled' device made from a transparent co-polymer of polyvinyl acetate and polyethylene. One tube contained a soft wire by which the dispenser was hung in the trees, the other contained *ca*. 70 mg of the active ingredient. Individual components of multi-component blends were not separated. (Fig. 9).

Bio-dispensers. These dispensers were made from the same polymeric material as the S-E dispensers but consisted of five individual tubes into which the components of a multi-component blend could be separately loaded. (Fig. 10).

Three adjacent tubes were loaded with EEOH and the remaining two with a 5:1 mix of 12OH:14OH. The total quantity of active ingredient was 600 mg.

Calculation of loss rates.

Shin-Etsu. Mean pheromone column length (in mm) at the previous reading was subtracted from the current mean, multiplied by 0.498* and divided by the number of elapsed hours between readings to give a loss rate for individual

dispensers in mg/hr. This rate was multiplied by the number of dispensers/ha to give the loss rate in mg/ha/h.

* 0.498 = the cross-sectional surface area of the pheromone column in mm.

Bio-dispensers. As for S-E dispensers except that the cross-sectional surface area was 1.41 mm^2. Losses were determined for the entire dispenser rather than for EEOH and the saturated alcohols separately.

6.4 Trap descriptions.

Delta traps. These traps were constructed from waxed-cardboard milk cartons. They were triangular in cross-section, with a base to apex height of 80 mm, base width of 95 mm and were 155 mm long (Fig. 11).

Their bases were coated with "tangle-trap" (The Tanglefoot Company, 314 Straight Avenue, S.W., Grand Rapids, Michigan). Alternatively, cardboard liners were coated with this material and secured to the trap base with paper clips.

Pheromone baits were placed centrally on the base of the trap.

Live traps. A hole was cut in the base of a delta trap to accommodate the rim (95 mm diameter) of a powder funnel, which was secured to the trap with tape. The neck of the funnel was placed inside a 750 ml translucent plastic canister, within which moths were trapped after passing through the funnel. (Fig. 12).

Pheromone baits were suspended in the middle of the funnel, just below the rim, and talc was applied to the inner surface of the funnel to improve trap efficiency.

Feeding lures. *ca* 2 l plastic buckets containing a terpinyl acetate/brown sugar solution to attract and trap adult codling moth. (Fig. 13). The recipe for the solution was as follows:

A.	Soft brown sugar	1 kg/10 l water
B.	Water	250 ml
	Terpinyl acetate	97 ml
	Tween 20	3ml

Add 15 ml of Mix B/10 l of Solution A.

Trap bands. Strips of corrugated cardboard *ca* 100 mm wide, stapled to the butts and major limbs of the trees to trap mature larvae. (Fig. 14).

6.5 Bait preparation.

A ten-fold dilution in toluene of the required quantity of pheromone (measured volumetrically) was dispensed with a Hamilton automatic syringe that delivered 10 µl of solution at a time into the lumen of 20 mm lengths of surgical rubber tubing. (i.d. 4.5 mm, wall thickness 1 mm). Baits were wrapped in aluminium foil and stored in a freezer at - 20°C until required.

6.6 Sources of pheromone and purity used in bait preparation and 'wheel' bioassays.

Note: Two values are given for purity (determined by gas chromatography). The first refers to the purity of material received from the manufacturers, the second to purity after distillation.

EEOH: Source: INRA (France). Isomeric purity 96.4%, >99.5%.

12OH: Source: Ajax Chemical, Sydney. >98%, >99.5%

14OH: Source: Aldrich Chemical Co. Milwaukee, Wisconsin. >98%, >99.5%.

E9-12OH. Source Shin-Etsu, Japan. Purity not determined.

6.7 Rearing technique.

The laboratory culture was maintained on apple thinnings which were gathered in December-January each year and stored in a cool room at *ca* 5°C until required.

All rearing took place in a room in which temperatures were maintained at 24°C ± 2°C, humidity within the range 55-65% and in a 14:10 l:d cycle.

Eggs were obtained by placing 3 females and 2 males in 360 ml translucent plastic drinking cups with ribbed sides (Detmold Packaging, No. 12T G 360) ('egging cups'). Cloth gauze was placed over the tops of the cups and secured with a rubber band to prevent the moths from escaping and a 5% sucrose solution was supplied through glass tubes stoppered with cotton wool wicks and inserted through the sides of the cups.

Egging cups were changed every 2-3 days, cut in half longitudinally and placed in a plastic bag with a moistened wad of cotton wool to maintain humidity. Within a day of the first eggs hatching the cups were distributed over apple thinnings placed in wire mesh trays, which in turn were housed within plastic boxes whose lids had largely been replaced with a fine stainless steel mesh to allow air circulation.

Beneath each tray and around the insides of the boxes strips of corrugated cardboard were placed as pupation sites. Pupae were removed from the cardboard and sexed. Those that were not required for maintenance of the culture were used for experimental purposes or otherwise stored for up to 2 weeks at 10°C, after which they were discarded.

Occasional introductions of field-collected pupae and adults were made in an attempt to maintain colony vigour. However to avoid the possibility of introducing the protozoan parasite *Nosema carpocapse*, known to be present in the wild populations from which material was drawn, eggs from all field-collected females were cool-stored until the female had died, after which she was examined for the presence of *N. carpocapse*. Only eggs from disease-free females were admitted to the culture.

Fig. 9. Shin-Etsu pheromone dispenser

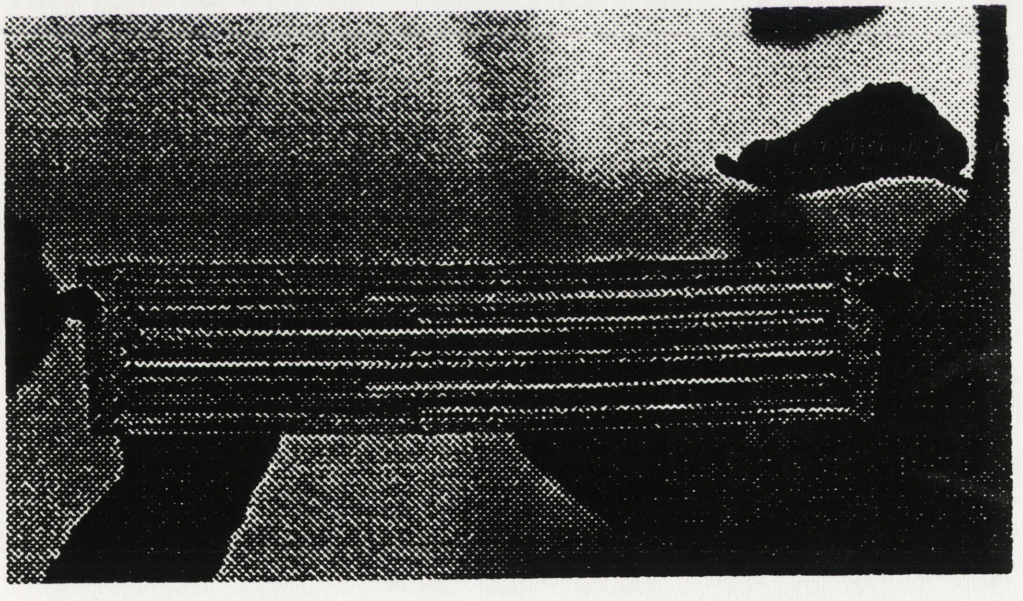

Fig. 10. Biodispenser.

Fig. 11. Delta pheromone trap.

Fig. 12. Terpinyl acetate feeding lure.

Fig. 13. Trap band for collecting mature larvae.